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TENSION DUE TO INTERACTION BETWEEN THE SLIDING FILAMENTS IN RESTING STRIATED MUSCLE. THE EFFECT OF STIMULATION

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SUMMARY

1. A resting sartorius muscle of the frog or toad possesses a special kind of elasticity which is shown to be due to a component lying between the two sets of filaments. The elastic effect is seen only for very small length changes, up to about 0·2 % of the muscle length, and the 'elastic limit' is then reached. If the length change then continues at a constant velocity the tension developed is maintained at a fixed level, producing a sort of frictional resistance. The component responsible is called the 'short-range elastic component', or SREC.

2. It is also shown that a small part of the permanent tension of a resting muscle is probably due to 'active' interaction between the filaments. This is called the 'filamentary resting tension', or FRT. For a sarcomere length of 2·0 μ the FRT amounts to about 150 mg in a muscle weighing 100 mg.

3. The stiffness of the SREC and the magnitude of the FRT are shown to be related to one another. They are both increased, and may rise to high values, by making the external solution hypertonic.

4. The working hypothesis is as follows. In a resting muscle the cross-bridges on the myosin filaments are not entirely inactive, but a very small proportion of them are cross-linked with the actin filaments. The links are very stable and have a long 'life'. The elastic behaviour is due to the flexural rigidity, or spring-like properties of these bridges. The elasticity is 'short-range' because the bridges can be bent, or stretched, only a small way from the steady-state position before the contacts 'slip'. The 'filamentary resting tension' (which is present in the absence of any external length change) is attributed to an 'active process', which operates by imparting 'organized' potential energy to the participating cross-bridges, by potentiating their attachment, against their elastic resistance, to sites on the actin filaments which are displaced towards the Z line.

5. It is shown that the ability of the muscle to maintain a 'frictional' resistance to a continuing, slow, length change is suppressed during a maintained tetanic contraction. The process of suppression starts during the period of the latency relaxation.

6. It is suggested that the latency relaxation may be due to a reduction of the filamentary resting tension.

7. The experiments with the latency relaxation were facilitated by the use of strongly hypertonic solutions. The positive twitch tension is then greatly reduced, or may be practically abolished, while the latency relaxation remains at about its normal size and is extended in duration.

INTRODUCTION

The investigation began with a study of a small, and at first sight somewhat insignificant, 'elastic' effect which may be elicited from a resting muscle. This form of elasticity had come to light, rather by chance, in the course of other work. The elastic property in question provides a simple, almost 'spring-like', resistance at the start of a change of length made even at a very low velocity. But only a very small length change is required before the 'elastic limit' is reached; the component responsible is therefore referred to as the 'short-range elastic component', or SREC. If the length change continues at a constant velocity after the 'elastic limit' is reached, the tension which has been developed in the SREC is maintained at a constant level, and this provides a sort of frictional resistance to further elongation.

It will be shown that the SREC is located between the two sets of sliding filaments.

The stiffness, or what will be called the 'elastic modulus' of the SREC is normally very small, but it can be greatly increased by soaking the muscle in hypertonic solutions: this ability to enhance the effect has greatly helped the investigation. It is shown that the behaviour of the SREC is modified as the result of stimulating the muscle, so that a slow elongation no longer produces tension in the SREC.

Only when the study of the SREC had been completed was it realized that another, related, property of the muscle had been overlooked. The stiffness of the SREC is measured by recording the change of tension resulting from a small length change. The stiffness increases when the solution is made hypertonic. It was not noticed, until later, that the permanent resting tension (on top of which the tension increment due to a length change was being measured) also rises when the solution is made hypertonic, and remains constant at the new level. The effect, like the change of stiffness of the SREC, is entirely reversed when the normal

solution is replaced. The part of the total resting tension which is capable of varying in this way has certain characteristics which show that it is probably due to some form of interaction between the filaments, and it has therefore been called the 'filamentary resting tension', or FRT. The FRT is only a small fraction of the whole resting tension of a muscle, in isotonic solution, when it is held at the normal length in the body; but in a muscle which is allowed to shorten passively to a length several mm less than the length in the body (to give a sarcomere length of 2·0 μ, which is the point at which the I-band filaments start to butt together in the middle of the A band) the FRT becomes an appreciable part of the whole tension, and amounts to about 150 mg in a muscle weighing 100 mg.

It is suggested that the latency relaxation may be due to a reduction of the FRT. The investigation of the nature of the latency relaxation has been greatly facilitated by the use of hypertonic solutions. As is well known, the twitch can be much reduced, or even abolished, by hypertonic solutions. It was found that the latency relaxation does not show any such reduction. By using hypertonic solutions it was possible to dissociate the tiny relaxation from the relatively enormous positive contraction of the isotonic muscle, and thus to study the relaxation in greater detail.

It is necessary to explain the use of terms such as 'elastic modulus', and 'frictional resistance'. It is suggested, as a working hypothesis (outlined in the Summary), that the elastic properties of the SREC are due to the mechanical stiffness of a small number of cross-bridges which make contact between the two sets of sliding filaments of a resting muscle. When the muscle is slowly stretched the strain on the cross-bridges is increased and the tension rises. If the velocity of length change is not too low the cross-connexions do not break spontaneously, or 'slip' under the added stress at a significant rate, during the early stages of the stretch, so the tension developed is then almost linearly related to the amount of stretch; hence the justification for the use of the word 'elasticity'. Later, when the length change approaches a certain limit (the 'elastic limit') the rate of slip, and the 'relaxation rate', increase rapidly and then, while the length change continues at the same velocity, the tension levels off to give a constant 'frictional resistance'. The analogy with a *frictional*, rather than with a *viscous* resistance is justified by the demonstration that the force developed is only slightly dependent on the velocity of the length change. The parameter referred to as the 'elastic modulus', or E, is the recorded stiffness of the SREC (expressed in the form of a Young's modulus) for an extremely small length change (much less than that required to stretch the component to the 'elastic limit') at a stated velocity of length change.

METHODS

A pair of sartorius muscles of the frog (*Rana temporaria*) or toad (*Bufo bufo*) was set up in the vessel shown in Fig. 1 *A*, *B*. The pelvic ends of the muscles were attached to an electromagnetic length transducer, operated from a generator giving linear or sinusoidal waveforms with variable amplitude and duration. The tibial ends of the muscles were

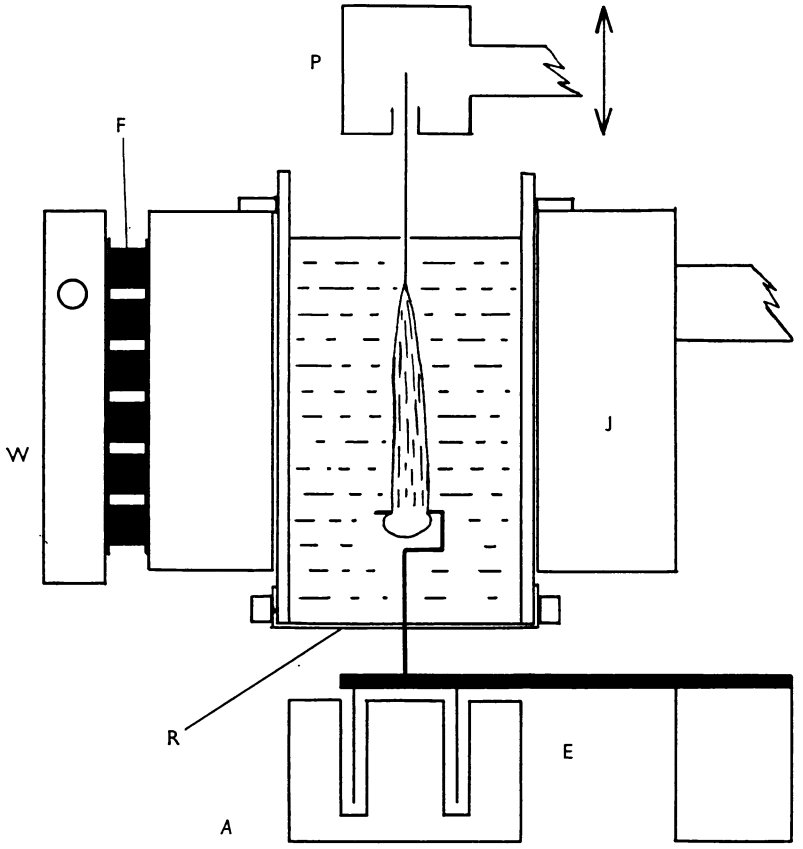

Fig. 1 *A*. For legend see opposite page.

attached to a photoelectric tension recorder. The tension was recorded on a cathode-ray oscilloscope with a Polaroid camera. For the experiments requiring only relatively slow recording but good baseline stability, the photoelectric recorder was replaced by a Statham strain gauge (Universal Transducing Cell, model UC2), and readings were made on a galvanometer.

Temperature control. Some of the experiments were done at room temperature, and others at a reduced temperature, generally 0° C. The cooling device is shown in Fig. 1 *A*, *B*. The muscle chamber was surrounded with an aluminium jacket. Efficient heat transfer was ensured by machining the aluminium block to give a close fit to the glass at room temperature, and with the lower expansion coefficient of the glass, the two were in contact over much of the area when cooled. The aluminium jacket was cooled by thermoelectric cooling modules (type 12–15 G, De La Rue Frigistor, Ltd., Maidenhead, Berks). The heat was

removed by water-cooled heat sinks. The current through the modules was switched on and off by a thermostat consisting of a thermistor probe and a control box for switching and selecting the temperature. An independent temperature-sensitive switch was fitted, to cut off the power in the event of a mains water failure (Probe, type D.13350/15/W/A; thermistor controller, type G. 535; Gravinette temperature control switch, type TC. S. 3150; available from Graviner Ltd., Colnbrook, Bucks.). A thermistor-operated thermometer was used for measuring the temperature of the solution in the muscle chamber. The thermostat maintained the temperature constant to within a fraction of 1° C.

Electromagnetic length transducer. This is a commercially available instrument (Advance Electronics Ltd., Vibration Generator, model V1) modified by replacing the compliant

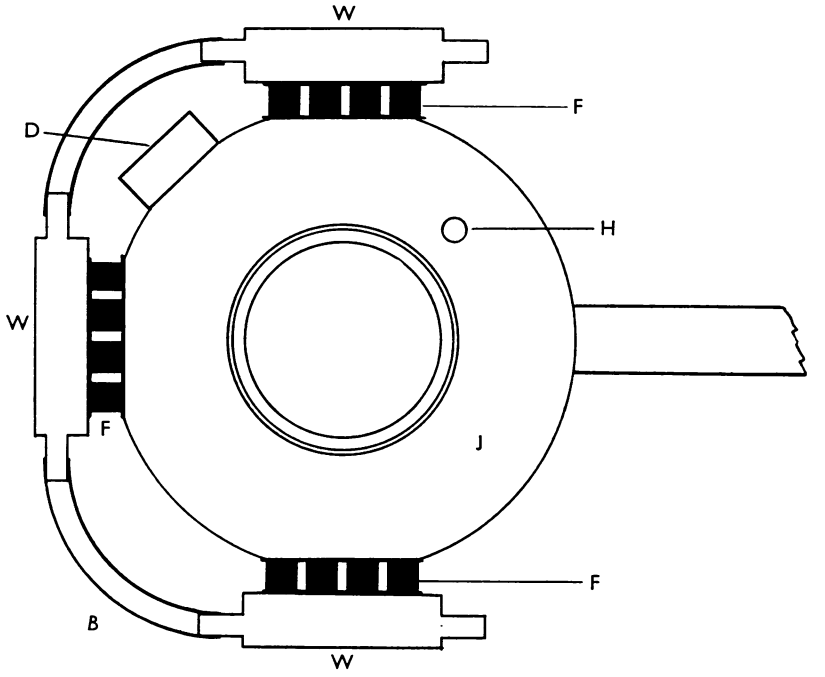

Fig. 1*B*

Fig. 1*A* shows a vertical section of the apparatus, and Fig. 1*B* is a horizontal section. Not to scale. A pair of sartorius muscles is held in Ringer solution in a cylindrical glass vessel, diameter 2·8 cm, height 7 cm, which is closed at the bottom with a rubber membrane, R. The pelvic bone is slipped over a platinum stirrup, the shaft of which passes through the rubber membrane and is connected with the electromagnetic length transducer, E. The tibial tendons are tied with cotton and connected by a steel hook, through a jeweller's chain, to the photoelectric recorder, P. (The Statham strain gauge may be used in place of the latter.) The length of the muscle is adjusted by raising or lowering the tension recorder. The solution is cooled, generally to 0° C, by three Frigistor thermoelectric cooling modules, F, which are clamped to an aluminium jacket, J (wall thickness, 13 mm). The heat is removed by the water-cooled sinks, W. The temperature is controlled by a thermistor probe which is inserted in a hole, H, in the aluminium jacket. A temperature-controlled switch, D, operating through a relay, acts as a safety device in the event of a failure of water pressure, by switching off the power to the cooling modules if the temperature rises above 40° C.

diaphragm by a stiff brass beam. The length of the beam was adjustable, and the two alternative settings gave the following performance characteristics: high frequency—linear response up to 150 c/s, compliance = 0·2 $\mu.g^{-1}$, maximum amplitude = 100 μ; low frequency—linear response up to about 15 c/s, compliance = 1·9 $\mu.g^{-1}$, maximum amplitude = 1050 μ.

The transducer was operated through a d.c. power amplifier from a waveform generator (Servomex Controls Ltd., Low Frequency Waveform Generator, type L.F. 141). Normally, a single-step linear (ramp) form was used, to give a change of length at constant velocity; for some experiments a triangular or sinusoidal oscillation was used. A transient length change could be superimposed on a continuous length change, the latter being provided by a motorized screw which raised or lowered the tension recorder. The length changes employed varied in amplitude from a few microns up to 1000 μ, with transit times of 200 sec, down to 8 msec for the smaller length changes (with the transducer beam in the high frequency position).

Photoelectric tension recorder. This was a stiff beam of stainless steel, 0·90 mm diameter, 13 mm long, compliance 1·8 $\mu.g^{-1}$, fitted with a shutter to modulate the light passing between a lamp and an aperture in front of a photomultiplier tube. The output of the photomultiplier was recorded on an oscilloscope. Any 'noise' of frequencies higher than those of the changes to be recorded was eliminated by a suitable filter.

The compliance of the system. The compliance of the photoelectric tension recorder (1·8 $\mu.g^{-1}$), together with that of the length transducer (0·9 or 1·9 $\mu.g^{-1}$), was generally negligible. The compliance of the Statham strain gauge was 2·0 $\mu.g^{-1}$, but with the lever attachment this figure was increased by a factor equal to the square of the mechanical advantage. The chain and hook which connected the muscles to the recorder added no further compliance. Nevertheless, the compliance of the whole system was in many cases far from being negligible when the tension was below a certain value, and the additional compliance was entirely due to the 'springiness' in the loop of cotton which had to be used for tying the tibial tendons to the hook at the bottom of the chain. Jewell & Wilkie (1958) have commented on the difficulty of avoiding this source of stray compliance. The total compliance, at a series of tensions, was measured (Table 1) by substituting a length of steel wire for the muscle, the wire being tied to the hook with cotton in the same way that the muscle normally would be.

A correction for instrumental compliance was made in working out the quantitative results.

Stimulation. The muscles were stimulated by passing single condenser discharges (10 μF, 100 Ω, time constant 1 msec) through the muscle from end to end (Fig. 1*A*). Repetitive stimulation, to give a maintained contraction, was provided by condenser discharges using a commutator, or by 50 c/s a.c.

Solutions. The Ringer solution had the following composition (mM): NaCl, 96; KCl, 5; $CaCl_2$, 4; sodium phosphates, pH 6·9, 5. The osmotic equivalent of this solution, calculated from the data given by Dydynska & Wilkie (1963), was 215 m-osmole/kg water. Hypertonic solutions were prepared by the addition of sucrose. The strengths of the solutions are expressed as grams of sucrose per 100 ml. of final solution, and are referred to in the form RnS, where n is the percentage of sucrose (g/100 ml.). The total osmotic equivalents of the Ringer–sucrose solutions were calculated from the formulae provided by Dydynska & Wilkie (1963) and the strengths, expressed in terms of the isotonic value as unity, are as follows: R, 1·0; R2S, 1·27; R4S, 1·56, R6S, 1·85; R8S, 2·16; R10S, 2·49; R12S, 2·82; R14S, 3·16; R16S, 3·51; R18S, 3·90; R20S, 4·29. (For instance, 6 % sucrose (w/v) in Ringer solution (R6S) has an osmotic pressure 1·85 times that of normal Ringer solution.)

In a few experiments the solution was made hypertonic by increasing the concentration of the normal ionic constituents of the Ringer solution.

The solutions were introduced into the muscle chamber by means of a syringe, through

a tube fixed over the rim of the vessel. The solutions were kept oxygenated via the same channel.

Muscle length and sarcomere length. The length of the sartorius before dissection, measured with the legs extended in line with the body, is denoted by l_0 and is referred to as the 'standard length'. Where it is necessary to make reference to the sarcomere length, this is estimated by assuming that it is equal to 2·4 μ with the muscle at the standard length. (A. V. Hill's 'standard length' is measured in a similar way, and Clinch (1965), using a light-diffraction method, measured the sarcomere length of a frog's muscle set at this length, and found it to be 2·36 μ. Sandow (1936) estimated the sarcomere length as 2·47 μ at the 'normal length' in the body.) The actual length will be referred to as l. The weight of the muscle, after equilibration with isotonic solution, is denoted by M.

TABLE 1. Compliance of apparatus with cotton attachment (units, $\mu.g^{-1}$)

	Photoelectric tension recorder					
			Statham strain gauge			
Tension (g)	(Transducer compliance 0·2 $\mu.g^{-1}$)	(Transducer compliance 1·9 $\mu.g^{-1}$)	No lever	× 2	× 5	× 10 lever
80–20	2	4	4	10	52	202
20–10	4	6	6	12	54	204
10–5	7	9	7	13	55	205
5	9	11	9	15	57	207
3	—	14	12	18	60	210
2	—	20	20	26	68	218
1	—	40	40	46	88	238
0·75	—	50	50	56	98	248
0·50	—	70	70	76	118	268
0·40	—	100	100	106	148	298
0·30	—	120	120	126	168	318
0·20	—	200	200	206	248	398
0·10	—	400	400	406	448	598

The tension P (or P_0 for the maximal tension in an isometric tetanic contraction) is generally given as the force (kg) per unit area of cross-section of the muscle (cm^2), where the latter is calculated as M/l_0 cm^2 (and was therefore not the actual value when the muscle was in hypertonic solution, or when l was not equal to l_0).

The stiffness, or 'elastic modulus' (E), of the SREC was measured by recording the tension change produced by a very small length change (p. 639). The result is given in the form of a Young's modulus: that is, it is expressed as the force which would be required, in kg, to double the length of the muscle, per cm^2 of cross-section.

RESULTS

The SREC in resting muscle

The characteristic response. The typical length–tension relation is shown in Fig. 2*A*. When the stretch starts, the tension rises rapidly at first, in an almost linear manner and with a slope which gives the value of the 'elastic modulus', E (defined on p. 639). The stiffness declines gradually during the subsequent rise, but then rather abruptly falls to zero, and the tension levels off at the maximum, or a little below, to give the so-called 'frictional resistance', which in this case is equal to 180 mg. The length

change for which the abrupt loss of stiffness occurs, 30 μ in this instance, is referred to as the 'elastic limit'. After the 'elastic limit' is passed the 'frictional resistance' is maintained so long as the length change continues. When it stops, the tension immediately starts to fall. The characteristics of the relaxation process will be considered later.

The value of E, for the experiment of Fig. 2*A*, corrected for the external compliance (using the values in Table 1) was 7·4 kg.cm^{-2}. This is small

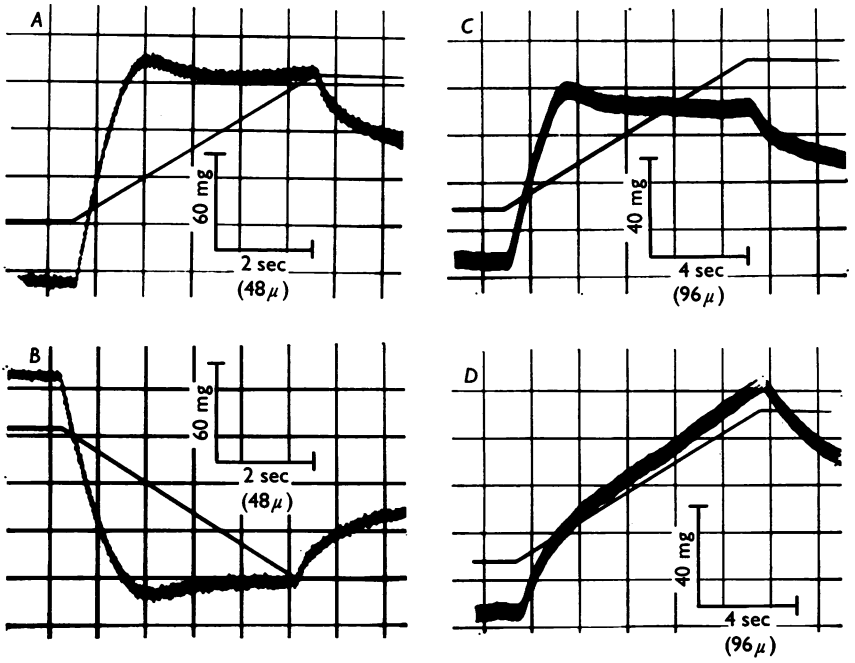

Fig. 2. The effect of a slowly applied stretch or release on the tension of a resting muscle. The tension was recorded (vertical axis) on an oscilloscope, with the beam sweeping continuously at constant speed. In each case the length change (made at constant velocity) started shortly after the beginning of the sweep at the point indicated by the ascent of the second trace (narrow) which registers the voltage applied to the length transducer: the length change ended at the point where the second trace levels off again. *A* and *B* show the characteristic length–tension relation for the SREC in slightly hypertonic solution (R3S). *C* shows the effect in isotonic solution (R) and *D* in hypotonic solution (0·5R). *A* and *B* are for a pair of frog's sartorii, l_o = 24 mm, l = 24 mm, M = 68 mg, 20° C. The total length change was +120 μ (stretch) for *A* and −120 μ (release) for *B*, at velocity 24 μ.sec.$^{-1}$. The tension at the start was about 2 g. The elastic modulus, E, is given by the slope of the length–tension record at the start. For *A*, E (corrected for compliance) = 7·4 kg.cm^{-2}. *C* and *D* are for a pair of toad's sartorii, l_o = 27 mm, l = 25 mm, M = 90 mg, 20° C. Length change = +240 μ, at velocity 24 μ.sec^{-1}. For *C*, E = 1·4 kg.cm^{-2}; for *D*, E (corrected for the elasticity of the 'parallel' elements, which is found from the slope of the final part of the record, before the length change ends) = 0·8 kg.cm^{-2}.

compared with the elastic modulus of the tendons and filaments (see below), so the two sets of filaments must be sliding past one another, and it follows that *the material responsible for the elastic response must be located between the filaments*. The sarcomere length in a sartorius at the standard length, l_0, is 2·4 μ, and a total length change of 30 μ is equivalent to 16 Å in each half sarcomere. This is the extent of the sliding movement of the filaments which generates the full tension in the SREC under the specified conditions.

The value of the elastic modulus of the tendons and filaments of a frog's muscle at a series of tensions has been estimated from information (unpublished) provided by A. V. Hill. The Young's modulus of the muscle, *plus the cotton connexions*, obtained from a force–length curve during a quick release of a contracting muscle (frog's sartorii; M = 0·15 g, l_0 = 30 mm P_0 = 100 g) was as follows: 162 kg.cm^{-2} (P = 100 g), 154 (90), 147 (80), 128 (70), 112 (60), 101 (50), 92 (40), 87 (30), 79 (20), 72 (15), 62 (10), 57 (7·5), 47 (5), 31 (2·5), 13 (1·0). At a tension of 2 g (this value is selected for comparison with the results of Fig. 2*A*, above) the elastic modulus is about 25 kg.cm^{-2}, and the compliance at this tension works out as 24 μ.g^{-1}. It will be seen from Table 1 that this is only a little greater than the compliance of the cotton connexion at the tension in question. (The type of cotton, and the method of tying the muscle, were similar in A. V. Hill's and in the present experiments, so it is reasonable to make use of these values.) It is clear, therefore, that the cotton is the main source of compliance at the lowest tensions. The filaments must have an elastic modulus which is not less than, say, 50 kg.cm^{-2}, at the tension in question.

Release compared with stretch. The length–tension relation during shortening (Fig. 2*B*) is almost exactly the reverse of what it is during lengthening.

An important point concerning the experimental procedure. In order to display a clear-cut length–tension relation, with an abrupt levelling-off of the tension at the 'elastic limit', it is essential that all the fibres shall generate tension at precisely the same time when the length change is applied. The only way of making sure that this happens is by subjecting the muscle to a preliminary length change in the same direction as that in which the test run is to be made: the muscle should be elongated by a few tenths of a millimetre before a test stretch is made, or shortened by a similar amount before a release. There must be a pause, after this 'conditioning' length change has been made, sufficient to allow the tension to settle to a steady level; about 30 sec was generally allowed.

The effect of 'parallel' elasticity at greater lengths. The 'parallel' elastic elements, which are responsible for most of the resting tension, are not concerned in the type of length–tension relation shown in Fig. 2*A*, *B*, because under the conditions of that experiment the elastic modulus of these 'parallel' elements is very small compared with that of the SREC. This is shown by the fact that the tension remains at a nearly steady value during a continuing stretch after the 'elastic limit' of the SREC has been passed. But if the 'parallel' elements are made stiffer by working at a greater initial length, the response is a composite one (Fig. 3). In hypotonic solution (Fig. 2*D*) the swelling of the muscle fibres brings into existence another type of 'parallel' elasticity, which presumably resides in the expanded sarcolemma.

The effect of hypertonic solutions. The 'elastic modulus', E, of the SREC in normal isotonic solution is only 1–2 kg.cm^{-2} (Figs. 2*C*, 3*A*). In hypotonic solution (Fig. 2*D*) it falls to an even lower value. When a muscle is put into a hypertonic solution, and time is allowed for osmotic equilibration, *E* is found to be raised. The stronger the solution the greater is the rise of *E*. When the concentration of sucrose exceeds about 10% (R10S) E rises to values approaching, or possibly exceeding, the elastic modulus of the series elastic component, and under such conditions it becomes impossible to estimate E accurately.

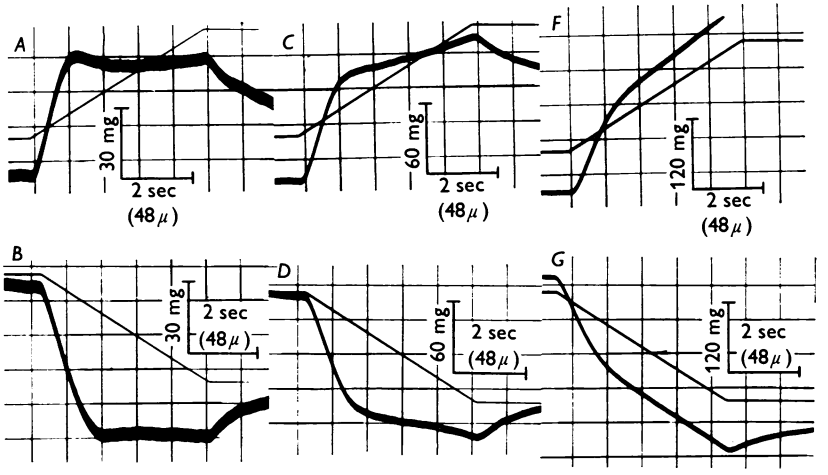

Fig. 3. The characteristic length–tension relation at different muscle lengths. Method of recording as for Fig. 2. Pair toad's sartorii, l_0 = 29 mm, M = 107 mg, 20° C, isotonic solution. *A*, *C* and *F* are for stretches of 120 μ; *B*, *D* and *G* are for releases of 120 μ. For *A* and *B*, *l* = 26 mm; for *C* and *D*, *l* = 29 mm; for *F* and *G*, *l* = 32 mm. For *A*, the elastic modulus E (defined as in Fig. 2) = 2·0 kg.cm^{-2}.

The time course of the change of E when the external concentration is raised. When frequent measurements of E are required, and it is not necessary to record the whole of the length–tension relation (as in Fig. 2*A*), the method employed is to apply a very small, oscillatory, length change, whose amplitude is much less than the length change required to take the SREC to its 'elastic limit', and then to measure the amplitude of the resulting tension oscillation. The oscillation can be left running indefinitely, so the change of E resulting, for instance, from the application of a solution with a different tonicity, can be followed continuously. An example is shown in Fig. 4. One feature, here, requires some comment. It is seen that the change of E takes place more rapidly when, for instance, the transition is from solution R9S to R12S, than it is from R to R6S. This is presumably due to the fact that the diffusion distances become shorter after osmotic shrinkage.

The SREC in depolarized muscle. When a muscle is depolarized by isotonic potassium sulphate the elasticity of the SREC is not affected.

The change of E with a penetrating solute. It can be shown that the effect of hypertonicity, when using sucrose, is not due to a change in the concentration of inorganic ions within the muscle fibres. This was done by raising the internal concentration of K^+ and Cl^- to high levels while at the same time maintaining a normal cell volume. Boyle & Conway (1941) have given the equations for calculating the composition of suitable solutions of potassium chloride and sucrose.

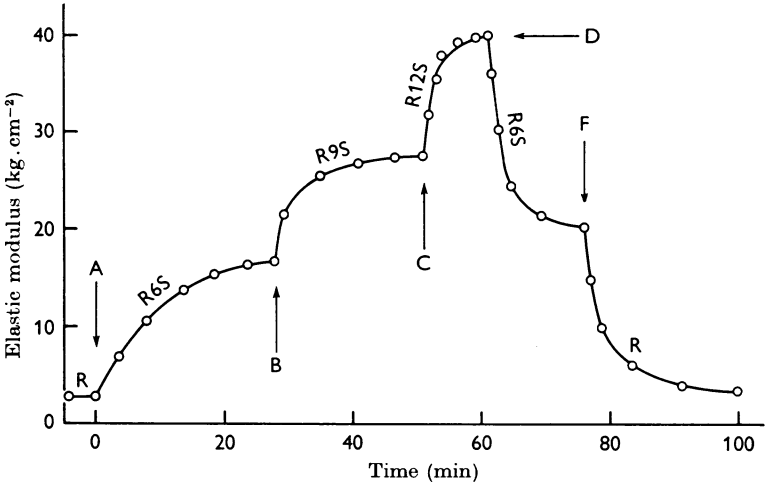

Fig. 4. Changes of elastic modulus, E, following immersion in hypertonic solutions. Pair frog's sartorii, l_0 = 26 mm, l = 25 mm, M = 110 mg, 20° C. E was measured by recording the amplitude of the tension oscillation due to a small sinusoidal length oscillation, amplitude 12 μ, frequency 20 c/s (the length–tension relationship can be regarded as linear for this length change). The solution was changed, at arrows, as follows: A, R to R6S; B, R6S to R9S; C, R9S to R12S; D, R12S to R6S; F, R6S to R.

The three conditions for equilibrium are as follows. (The suffix i denotes 'internal', and o 'external'.)

(1) Osmotic equilibrium: $(A/x)+[K_i^+]+[Cl_i^-]$ = external osmotic equivalent, where A is the concentration of internal diffusible anions at normal osmotic pressure, and x is the final volume of the fibre water, expressed as a fraction of the normal volume.

(2) Donnan equilibrium: $[K_i^+]\times[Cl_i^-] = [K_o^+]\times[Cl_o^-]$.

(3) Electroneutrality: this requires that the net change of $[K_i^+]$ and of $[Cl_i^-]$ shall be equal, that is $[K_i^+]-[Cl_i^-] = D/x$, where D is the difference between the internal potassium and chloride concentrations at normal osmotic pressure.

In a normal muscle in isotonic solution $[K_i^+]$ = 139 mg-ion.l^{-1}. (Adrian, 1956); $[Cl_i^-]$ = 4 mg-ion.$l.^{-1}$ (Adrian, 1961), giving D = 135 mg-ion.$l.^{-1}$. The normal osmotic pressure is equivalent to 240 mg-ion.$l.^{-1}$. A = 97 mg-ion.$l.^{-1}$. Sucrose at 7·5 % is taken as isotonic.

The calculated values of $[K_i^+]$, $[Cl_i^-]$ and of x, for several KCl/sucrose mixtures are given in Table 2.

The changes in the elastic modulus, E, following the application of KCl/sucrose solutions are shown in Fig. 5. At the start of the experiment the effect of R7·5S is shown as a standard for comparison (fibre water, $x = 0{\cdot}5$); this is followed by KCl + 7·5S. The most striking demonstration of the point in question follows the immersion in 2KCl + 3·75S; here there is a small increase of E at the start, but this is soon followed by a fall, while the KCl penetrates the fibres, and E finally comes down to the value found in isotonic solution. Lastly, application of 5KCl + 7·5S gives a very large early increase, but this again shows a return to a lower value.

TABLE 2. The calculated internal potassium and chloride concentrations, and the volume of the fibre water, in a muscle equilibrated with a series of KCl/sucrose solutions

Composition of solution	$[K_i^+]$ (mg-ion.l.$^{-1}$)	$[Cl_i^-]$ (mg-ion.l.$^{-1}$)	x (fibre water)
Normal Ringer	139	4	1
KCl + 7·5S	271	52	0·62
2KCl + 7·5S	395	146	0·54
2KCl + 3·75S	320	180	0·97
4KCl + 15S	790	291	0·27
4KCl + 11·25S	717	321	0·34
5KCl + 7·5S	760	474	0·47

nKCl denotes KCl at n times isotonic concentrations; nS denotes n % sucrose, w/v.

This shows that the increase of elastic modulus of the SREC in a hypertonic solution is associated with the change of fibre water rather than with the internal concentration of ions. In 2KCl + 3·75S, $[K_i^+]$ is at 2·3 times its normal value, and $[Cl_i^-]$ is greatly raised; yet in spite of this the elastic modulus returns to its normal value; so also does x, the measure of fibre volume.

It can be concluded, therefore, that it is either the transverse distance between the filaments, or the degree of concentration of the non-ionic sarcoplasmic solutes which counts; or both factors may be concerned. The matter is considered again later.

The velocity of length change. The importance of velocity in determining the parameters of the elastic response of the SREC was investigated. The relation between E and the velocity is shown in Fig. 6 (lower curves). E is seen to change very little at the lower end of the range; in this instance it increases only from 9 to 23 kg.cm^{-2} for a 10,000-fold change of velocity. Fig. 6 also shows the length change required to stretch the SREC almost to its 'elastic limit'. The maximal tension developed during a continuing length change (that is, the height of the 'plateau' of the length–tension relation, as in Fig. 2*A*) at different velocities is shown in Fig. 7. At the lower velocities this parameter increases only five times for a 1000-fold increase of velocity.

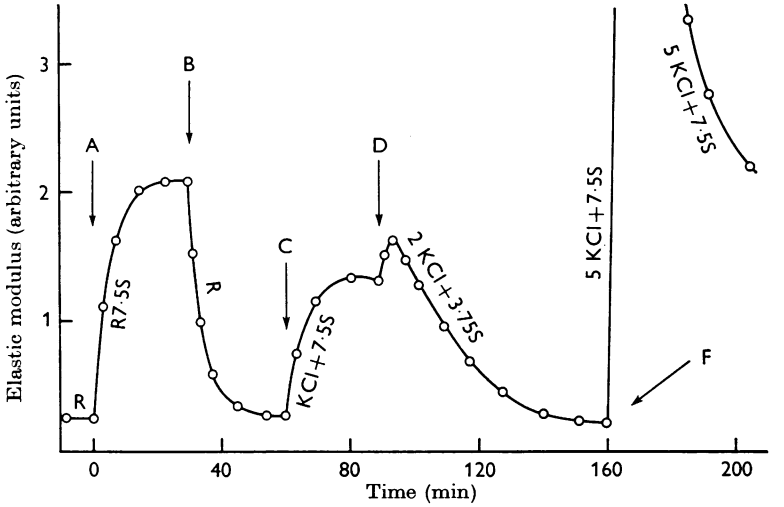

Fig. 5. Changes of elastic modulus, E, to show reversal following immersion in hypertonic solutions containing KCl. Pair frog's sartorii, l_0 = 24 mm, l = 23 mm, M = 80 mg, 20° C. E was measured (arbitrary units) by recording the amplitude of the tension oscillation due to a small sinusoidal length oscillation, amplitude 15 μ, frequency 20 c/s (the length–tension relationship can be regarded as linear for this length change). The solution was changed, at arrows, as follows: A, R to R7·5S; B, R7·5S to R; C, R to isotonic KCl for 2 min, then (after the contracture had subsided) to KCl+7·5S; D, KCl+7·5S to 2KCl+3·75S; F, 2KCl+3·75S to 5KCl+7·5S.

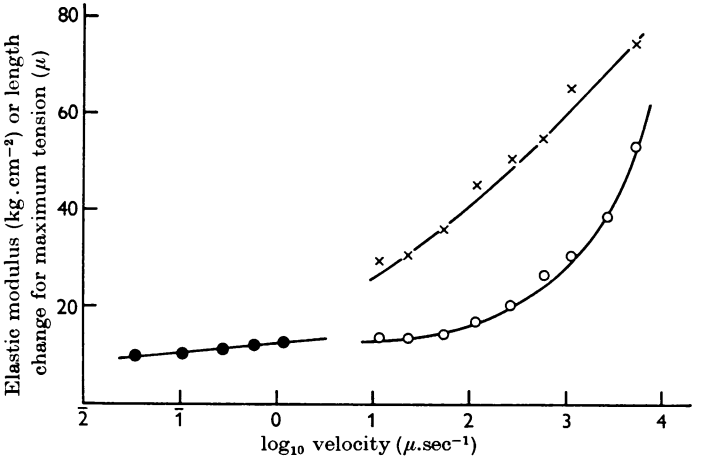

Fig. 6. The elastic modulus, E, at different velocities of length change (○ and ●). The modulus was measured by recording the tension oscillation due to a triangular-form length oscillation, of amplitude 10 μ, at a series of frequencies. Also (shown by ×), the length change required to stretch the SREC almost to its 'elastic limit' (this length change was arbitrarily selected as that at which the elastic modulus in the complete length–tension relation (e.g. Fig. 2*A*) had fallen to one quarter of E). The records ○ and × are for a pair of frog's sartorii, l_0 = 26 mm, l = 26 mm, M = 116 mg, 20° C, solution R6S. Record ● is for a pair of frog's sartorii, l_0 = 26 mm, l = 24 mm, M = 95 mg, 20° C, solution R4S.

The demonstration that the 'elastic modulus', and the maximal or 'frictional' resistance during a continuing length change, are very far from being linearly dependent on the velocity leads to the conclusion that viscous forces are not primarily concerned in determining the behaviour of the SREC.

The relaxation rate: its non-linear dependence on the tension. The relaxation rate is not proportional to the tension in the SREC. If it were, the 'characteristic response' (e.g. Fig. 2*A*) would have an exponential form. In fact, the early rise of tension is almost proportional to the length change, and the tension later levels off rather abruptly. It is clear that the relaxation 'rate constant' increases as the tension rises. One striking illustration of this is given in Fig. 8, which shows the effect, on the tension response to a small length change at high velocity, of being superimposed on a continuous length change at a much lower velocity. The relaxation 'rate constant' was greatly increased by this procedure.

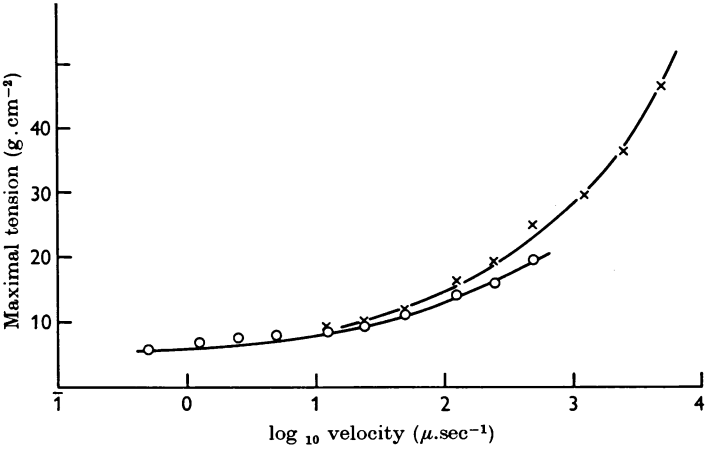

Fig. 7. The maximal tension developed at different velocities. Record ○ is for a pair of frog's sartorii, l_0 = 28 mm, l = 26 mm, M = 105 mg, 20° C, solution R4S. Record × is for a pair of frog's sartorii, l_0 = 26 mm, l = 24 mm, M = 116 mg, 20° C, solution R6S. The tensions were recorded during stretches of 120 μ, such as that shown in Fig. 2*A*.

The dependence of E on the muscle length. The SREC is located between the two sets of sliding filaments, and since the overlap region decreases in length when a muscle is stretched it was expected that E might also be found to decrease with stretch. It was not possible to make the test over a sufficient range of lengths when using isotonic or slightly hypertonic solutions, because of the difficulty of making an accurate allowance for the elasticity of the 'parallel' elastic elements (which is estimated from the slope of the 'long-range' length–tension curve, e.g. Figs. 9, 10). However, in solution R8S, or above, reliable figures were obtained, and some results for a series of lengths corresponding to a change of sarcomere length from 2·0 to 3·0 μ are given in Table 3. These show, contrary to expectation, that

there is rather little change of E with length: the length of the overlap region is reduced about three times for an increase in sarcomere length from 2·0 to 3·0 μ (Page & Huxley, 1963). It must be concluded, therefore, that the elastic modulus *per unit length of overlap* increases when a muscle is stretched. This is presumably due to the fact that the transverse distance

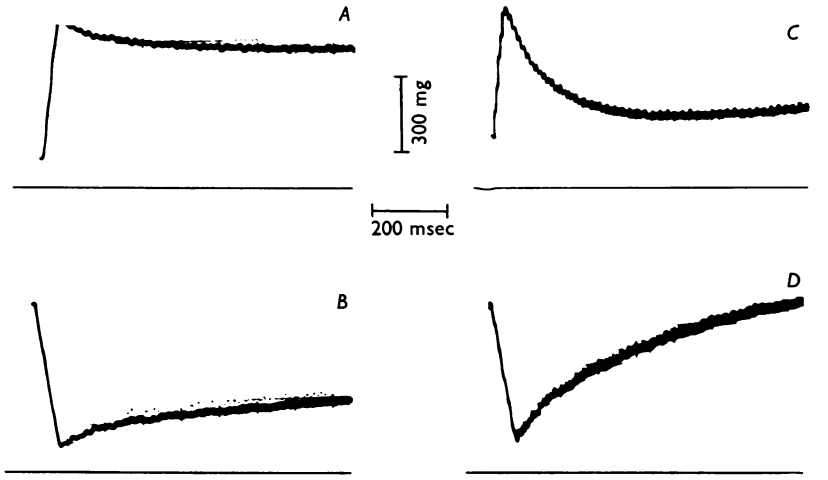

Fig. 8. Records *A* and *B* show the effects of a 30 μ stretch (*A*) and of a 30 μ release (*B*) at high velocity. Owing to an error in setting the speeds, the stretches were twice as fast as the releases, the times being 40 msec (velocity, 750 $\mu.sec^{-1}$) for the stretches, and 80 msec (velocity, 375 $\mu.sec^{-1}$) for the releases. *C* and *D* show the effects of the same short, rapid, stretch or release when these were superimposed upon a continuously running elongation (*C*), or shortening (*D*) at a much lower velocity, 50 $\mu.sec^{-1}$. Pair frog's sartorii, l_0 = 27 mm, l = 27 mm, 20° C, solution R5S.

TABLE 3. The elastic modulus of the SREC at different muscle lengths

Length of muscle (mm)	Elastic modulus (E) ($kg.cm^{-2}$) Expt. 1	Expt. 2
20	—	24·5
21	10·6	25·5
22	10·3	25·0
23	10·2	25·4
24	10·2	25·8
25	10·7	25·9
26	10·5	24·9
27	11·2	25·5
28	11·4	24·7
29	11·6	23·9
30	11·5	—
31	10·3	—

Expt. 1. Pair frog's sartorii, l_0 = 25 mm, M = 94 mg, 0° C, R8S. Expt. 2. Pair frog's sartorii, l_0 = 24 mm, M = 61 mg, 0° C, R10S. E was measured by recording the amplitude of the tension oscillation due to a length oscillation, amplitude 9 μ, frequency 0·5 c/s. Correction was made for instrumental compliance and for the elastic modulus of the 'parallel' elastic components.

between the filaments decreases. (The effect is even more marked with the FRT; it will be shown later that the measured value actually increases when the muscle is stretched.)

The dependence of E on the concentration of the sarcoplasmic solutes. It was shown, above, that the effect of hypertonicity in increasing E could be due to an increase in the concentration of the sarcoplasmic solutes, other than K^+ or Cl^-, or to the transverse distance between the filaments, or to both factors operating together. It has just been seen that the elastic modulus, per unit length of overlap, is increased by elongating the muscle; this is probably due to a reduction of the gap between the filaments. But it can also be shown that the concentration of the sarcoplasmic solutes is an important factor. It will be assumed, in what follows, that the filament lattice maintains a constant volume at different lengths. Experimental evidence for this was obtained from muscles in isotonic solution (Huxley, 1953; Elliott, Lowy & Worthington, 1963). It may not be strictly true in a hypertonic muscle, but a minor deviation from the constant–volume relationship would not invalidate the following conclusion. By a suitable adjustment of the muscle length it was possible, by calculation, to set the same filament spacing at two different osmotic pressures. The elastic moduli under the two sets of conditions were then compared. The following is an example. A pair of frog's sartorii (l_0 = 30 mm, M = 133 mg, 0° C) was set in isotonic solution at l = 33 mm (sarcomere = 2·65 μ). E, for a velocity of 270 μ.sec^{-1}, was 0·61 kg.cm^{-2}. This is 0·64 kg.cm^{-2} per micron of overlap. (In this calculation, the A-band filaments are assumed to be 1·6 μ long, and the I-band filaments 2·1 μ; these are the values given by Page & Huxley, 1963.) It was calculated, from the figures provided by Blinks (1965) giving the volume change of a muscle fibre resulting from an increase of osmotic strength of the external solution, that the same lateral spacing between the filaments should occur at a muscle length of 25 mm (sarcomere = 2·0 μ) in a solution of strength R5S. At l = 25 mm, and in R5S, E was found to be 15·3 kg.cm^{-2} per micron of overlap. So it is seen that the elastic modulus, per unit length of overlap, increased twenty-five times as the result of osmotic shrinkage, even though the filament spacing was the same in the two cases. It is concluded, therefore, that the increase in concentration of the non-ionic sarcoplasmic solutes must have been responsible for the increase in elastic modulus, and that this factor is very important in controlling the elastic effects of the SREC.

The filamentary resting tension

A resting muscle exerts measurable tension down to 60–75 % of its 'standard length' in the body (A. V. Hill, 1949). In Fig. 9 (lower) the resting tension is shown for lengths down to 79 % of the 'standard length'.

It was found that when the external solution was made hypertonic the resting tension increased (Fig. 9, upper). The well-known 'hysteresis' effect, observed when performing a length–change cycle (Fig. 9), will be referred to later. The immediate point to which attention is drawn is that even the lower part of the hysteresis loop for the muscle in hypertonic solution lies above the upper part of the loop for the isotonic muscle. It is therefore clear that the permanent resting tension has been increased by making the solution hypertonic. A further indication that the effect is due to a real increase in 'equilibrium' tension is provided by experiments, described later, which show that the tension *at a fixed length* increases when the tonicity is raised.

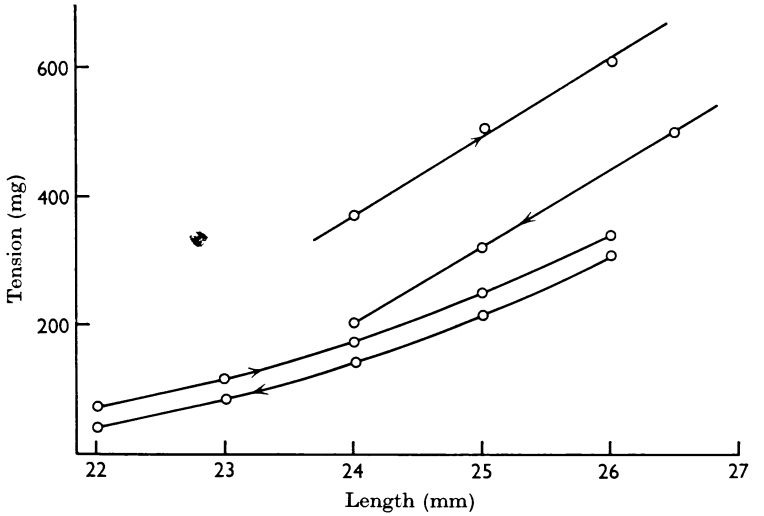

Fig. 9. The tension–length relation and hysteresis in resting frog's muscle in normal solution (below) 1 min after setting length, and in solution R4S (above) 5 min after setting the length. Pair frog's sartorii, l_0 = 28 mm, M = 108 mg, 20° C. The arrows show the directions of length change.

It is not impossible that hypertonicity may produce some rise in tension of the 'parallel' elastic components, but it is unlikely that this is the chief cause of the tension increase. The point is best explained by reference to Fig. 10: it is seen that the increase of tension with hypertonicity changes rather little with length (though there is, in fact, a small dependence on length, and this is considered later). On the other hand, the slope of either curve increases rapidly with a length increase. Therefore, the amount of tension increase with hypertonicity does not depend on the 'long range' elastic modulus of the muscle, which must be assumed to be largely due to the 'parallel' components. If the rise of tension with hypertonicity were caused by some change in the mechanical properties of the 'parallel' com-

ponents, the size of the effect could hardly fail to show a change with length which was similar to the change of the elastic modulus of these components.

The extra tension is still present at muscle lengths down to those at which the I-band filaments come together in the middle of the A band ($l = 0{\cdot}83\ l_0$ corresponds to a sarcomere length of $2{\cdot}0\ \mu$, which is the length of the I-band filaments—Page & Huxley, 1963) so the S filaments, earlier postulated (Huxley & Hanson, 1954; Hanson & Huxley, 1955) as joining together the ends of the I filaments across the H gap, cannot be involved.

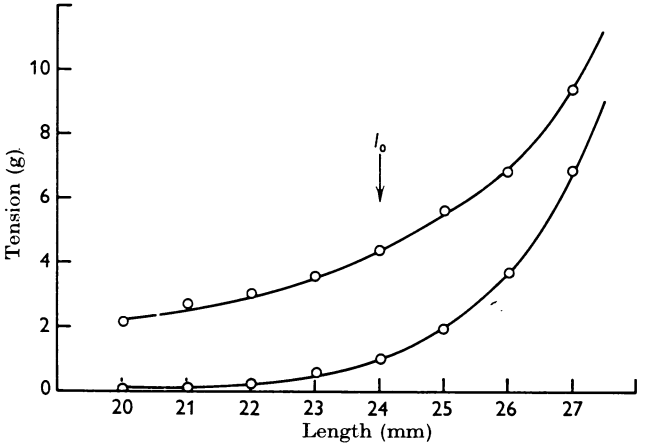

Fig. 10. Tension–length relation of resting frog's muscle in normal solution (below) and in solution R10S (above). Pair sartorii, l_0 = 24 mm, M = 61 mg, 0° C. The records were made with increasing length. The tension was measured 3 min after setting the length.

Another reason for thinking that hypertonicity is not acting primarily on the 'parallel' components is provided by the demonstration (see below) that an important factor in controlling the amount of extra tension is a change of the fibre volume, rather than of the tonicity *per se*. The only 'parallel' component which would be affected by fibre volume, rather than by the strength of the medium, would be the fibre membrane, but this would become less stiff, and show less tension, when shrunken by hypertonicity, not the reverse.

It is therefore fairly certain that the extra tension has its origin in some form of interaction between the two sets of sliding filaments. Hence the adoption of the expression 'filamentary resting tension' (FRT).

Any *increase* of tension, due to hypertonicity, may be called FRT, but the question remains: what is the amount of FRT in isotonic muscle? E can be measured at any one tonicity, but the FRT cannot be measured separately, because it cannot be distinguished from the other forms of

tension. But if the assumption is made that the FRT remains quantitatively proportional to E when the tonicity of the solution is changed (as it should be if the hypothesis to explain the origin of the effects is accepted) then the magnitude of the FRT in isotonic solution can be found. The method is described later.

The FRT at different lengths. The extra FRT developed in a hypertonic solution is greater when the muscle is longer. This is seen clearly in Fig. 9; in Fig. 10, though the effect is less marked, the vertical distance between the curves gets greater when the length increases from 20 to 25 mm. There is difficulty, in studying the effect of length, in making allowance for the large tension developed in the 'parallel' components at the greater lengths. In Fig. 10 the extra FRT apparently starts to decrease when the length is greater than about 25 mm: a possible explanation is that the contribution to the total tension by the sarcolemma was reduced by the osmotic shrinkage.

Since the amount of filament overlap decreases when the muscle is stretched, it follows that the extra FRT contributed by each micron of overlap must be increasing fairly steeply when the muscle is elongated. The same is presumably true of the whole FRT.

Hysteresis. When a muscle was taken through a cycle involving a change of length of several millimetres a substantial 'permanent' hysteresis tension difference was always found (Fig. 9), and this difference was greater at the higher tonicities. For a very short cycle, involving length changes not much greater than those required to produce maximal tension in the SREC, the permanent difference between the tensions measured after a stretch and after a release to the same final length, was very much smaller. These results suggest that the FRT shows some hysteresis as the result of a *large* length change. On the other hand, it is not likely that the whole of the hysteresis (Fig. 9) can be attributed to the FRT. The 'parallel' and 'series' elastic components are probably concerned too. An increase of the hysteresis from the 'series' component could be due to the greater tension changes required in overcoming the higher resistance of the SREC in the hypertonic muscle.

The time course of the change of resting tension following a change of tonicity. An example is shown in Fig. 11. The muscle was held at a constant length throughout. The spontaneous generation of tension under these 'static' conditions shows the 'active' nature of the process involved. An indication of the 'steady-state' nature of the final condition is the fact (not illustrated in Fig. 11) that the tension reached at a given concentration is maintained constant indefinitely, and that no 'relaxation' takes place.

In the experiment of Fig. 11 the recorded tension in R15S was 5 g, or 0·12 kg.cm^{-2}. This is as much as 8% of the tension produced in an isometric contraction in isotonic solution. In other experiments even higher maxima have been recorded. There is difficulty in obtaining reliable results at the highest concentrations because, as already seen, the stiffness of the SREC rises to very high values, and the system becomes extremely

sensitive to very small length changes such as may be produced, for instance, by small variations in the temperature of the apparatus.

Although high tensions may be produced, an unstimulated muscle in hypertonic solution is not able to generate an appreciable amount of mechanical power, or do work. The resistance of the SREC is such that if a muscle in R15S is released, even by only a few tenths of a millimetre, tension is regained only very slowly over a period of many minutes, and never returns to its original value. In the same connexion, it should be pointed out that although it may be true that metabolic energy is needed to maintain the FRT, it does not follow that the amount of energy needed is necessarily greater in a hypertonic muscle, because a higher tension would be maintained with the same expenditure of energy if the process of tension maintenance became more efficient. There are further comments on the 'energetics' of the process in the discussion.

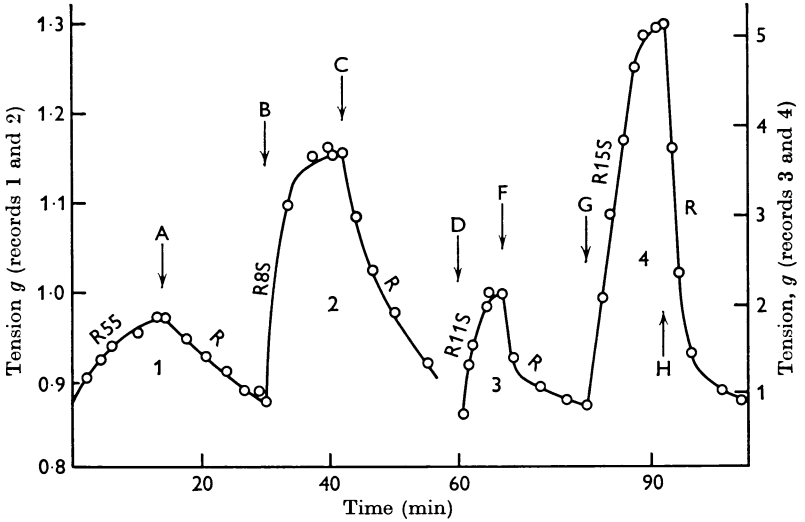

Fig. 11. The change of resting tension due to changes of the tonicity of the solution. Pair frog's sartorii, l_0 = 26 mm, l = 24 mm, M = 110 mg, 20° C. The tension at the start, in normal solution, was 0·87 g. At time zero normal solution was replaced by solution R5S. Thereafter the solutions were changed as follows: A, R5S to R; B, R to R8S; C, R8S to R; D, R to R11S; F, R11S to R; G, R to R15S; H, R15S to R. Note the change of vertical scale after record 2.

The change of tension with a penetrating solute. The changes following the application of KCl/sucrose solutions are shown in Fig. 12. The result is similar to that obtained with the SREC, and need not again be discussed in detail. It shows that the internal concentrations of K^+ and Cl^- are not concerned in controlling the magnitude of the FRT.

The factors controlling the magnitude of the FRT. It has already been seen that the FRT, per unit length of overlap, is increased by lengthening the muscle; this is probably due to a reduction of the distance between the two sets of filaments. It can also be shown that the concentration of

sarcoplasmic solutes is an important factor: the proof of this is exactly similar to that put forward, above, in connexion with the SREC.

The magnitude of the FRT in a normal muscle. It has been pointed out, above, that an indirect means has to be employed to measure the *total* magnitude of the FRT, or to make an estimate of its value in isotonic solution. The assumption was made that the FRT may be taken as being proportional to the elastic modulus, E, and the FRT of isotonic muscle was then estimated as follows. The resting tension and E were measured in

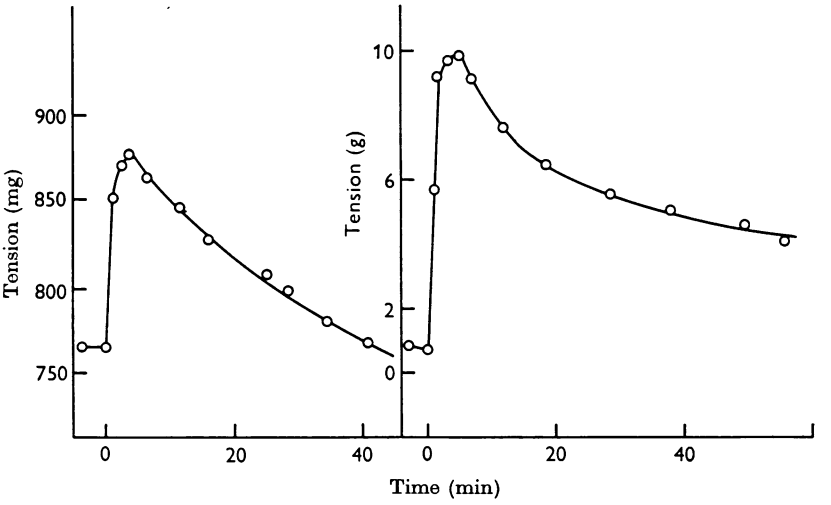

Fig. 12. Experiment to show that the increase of resting tension caused by hypertonic sucrose/KCl solutions is not maintained. Pair toad's sartorii, l_0 = 26½ mm, l = 22 mm, M = 70 mg, 20 °C. First part: at time zero the solution was changed from KCl + 7·5S to 2KCl + 3·75S. Second part: at time zero the solution was changed from 2KCl + 3·75S to 5KCl + 7·5S. Details concerning the solutions are given in Table 2.

R4S and also in isotonic solution. Suppose the results were: R4S, tension = A, E = B; isotonic solution, tension = C, E = D. An increment of FRT = A − C corresponds to an amount of E = B − D. The magnitude of the FRT in isotonic muscle, where E can be directly measured, can therefore be taken as $D \times (A - C)/(B - D)$. The results of eight experiments are given in Table 4. They are not very consistent. There was a threefold variation in the estimated amount of FRT per unit of E; but the FRT, expressed as g.cm^{-2}, showed a sixfold variation over the series. Part of the inconsistency can be attributed to the usual difficulty of making an accurate correction for the instrumental compliance when calculating the value of E. The mean value for the FRT in isotonic solution was 3·94 g.cm^{-2}, or 157 mg for a pair of sartorius muscles of weight 100 mg

TABLE 4. Estimate of the FRT of a muscle in hypertonic solution

Animal	Weight of pair sartorii (mg)	l_o (mm)	l (mm)	Tension in isotonic solution ($g.cm^{-2}$)	E in isotonic solution ($kg.cm^{-2}$)	Tension in hypertonic solution (R4S) ($g.cm^{-2}$)	E in hypertonic solution (R4S) ($kg.cm^{-2}$)	FRT ($g.cm^{-2}$) per unit E ($kg.cm^{-2}$)	FRT ($g.cm^{-2}$)
Toad	57	25	20	10·4	2·79	21·9	6·85	2·83	7·90
Frog	181	32	28	8·6	1·07	14·5	3·27	2·70	2·90
Frog	264	35	31	6·1	1·01	11·1	3·56	1·98	2·00
Toad	72	23	20	19·0	1·27	24·0	3·00	2·89	3·67
Frog	119	41	36	21·1	2·00	26·0	3·53	3·20	6·40
Frog	122	35	31	18·8	1·21	22·5	2·02	4·57	5·53
Frog	113	27	23	15·8	1·18	19·1	3·69	1·48	1·75
Frog	111	26	22	15·3	1·33	18·7	4·60	1·04	1·38
				Mean 14·7					Mean 3·94

E was measured by recording the amplitude of the tension oscillation produced by a sinusoidal length oscillation (amplitude 27 μ, frequency 0·1 c/s), with correction for compliance. Temperature, 20° C.

and l_0 = 25 mm. The mean FRT was 27 % of the mean total resting tension. The muscle lengths were 85 to 90 % of the standard lengths, giving a sarcomere length of about 2·1 μ.

In discussing the physiological significance of the FRT it is pointed out, later, that the FRT may be required to pull together the two sets of filaments of a passively shortened muscle. In order to be able to do this at a speed sufficient to be useful to the animal, say at not less than 0·1 mm.sec^{-1}, the FRT would have to be at least comparable with the 'frictional resistance' at this speed of length change. The 'frictional resistance' was not measured in the experiments of Table 4, but it may be approximately deduced as follows. From Table 4 it is seen that the FRT is equal to 2·59 g.cm^{-2} for E = 1 kg.cm^{-2}, at a velocity of about 5 μ.sec^{-1}. E does not increase appreciably with velocity up to 0·1 mm.sec^{-1} (Fig. 6). The 'frictional resistance' is developed for a length change equal to about 0·2 % l_0 at a velocity of 0·1 mm.sec^{-1}, and allowing for the progressive decrease in the stiffness of the SREC when the 'elastic limit' is approached (Fig. 2), the effective distance for which the E value operates may be taken as 0·15 % l_0. Thus the FRT is 2·59 g.cm^{-2} for a 'frictional resistance' equal to 1·5 g.cm^{-2} at a velocity 0·1 mm.sec^{-1}. The FRT would therefore certainly be able to overcome the 'frictional resistance' of an isotonic muscle at this velocity, and the limit would probably be more nearly 1 mm.sec^{-1}.

Temperature

The stiffness and tension of an inert biological tissue, where no chemical reactions are involved in producing tension, vary with temperature in a way which is fairly well understood. In the present case, however, the problem is more complicated. According to the working hypothesis (see Summary) the effects which have been described are due to the cross-bridges, which, even in the resting muscle, make occasional contacts with the actin filaments. Two processes are thought to be involved, one of which 'makes' the contacts, and the other 'breaks' them. The elastic behaviour of the SREC, and the magnitude of the FRT, therefore represent a balance between two opposing processes. If both processes have rates which increase when the temperature rises, as is likely to be the case, the net result of a temperature change is unpredictable. In fact, it has been shown that the sign of temperature-dependence of E, and also that of the total resting tension, actually reverse when the tonicity of the external solution is changed. Unfortunately, the FRT cannot here be distinguished from the total tension, but it seems reasonable to conclude that both E and the FRT have a reversible temperature-dependence. This is consistent with the idea that they depend on a balance between two opposing processes, and that the effects are not due to some inert elastic material. The result therefore gives some support to the working hypothesis.

Temperature change and the stiffness of the SREC. The temperature-dependence of E is positive when the muscle is in isotonic solution (Table 5). This is apparent only when the muscle is at a length less than that at which the 'parallel' elastic elements become dominant: at greater lengths, the increasing importance of 'normal' elastic characteristics (A. V. Hill, 1952) causes the sign of the temperature-dependence to reverse. In slightly hypertonic solutions the temperature-dependence becomes smaller, or negligible, and in more strongly hypertonic solution it reverses and becomes negative.

Temperature change and the resting tension. There is no way of determining the temperature-dependence of the FRT itself, because it is impossible to make any reliable assumption about the temperature-dependence of the other forms of tension which make up the total.

The total resting tension was found to be positively temperature-dependent in isotonic solution, but it became negatively dependent at a sufficiently high external tonicity (Table 6).

Experiments were made to show how the tension varies during the progress of a slow, con-

TABLE 5. The dependence on temperature of the elastic modulus

1. *Isotonic solution.* $l = 1{\cdot}03l_0$ (SREC dominant). The measurements of elastic modulus were taken in the following sequence: 2·60 (22° C), 2·06 (6° C), 1·99 (6° C), 2·60 (23° C), 2·00 (6° C), 2·03 (5° C), 2·60 (26° C), 2·04 (6° C). Mean values: 24° C = 2·60; 6° C = 2·02. The elasticity has a substantial *positive* temperature dependence.

2. *Isotonic solution.* $l = 1{\cdot}14l_0$ (parallel elasticity dominant). 2·48 (5° C), 2·18 (26° C), 2·24 (6° C), 2·04 (27° C), 2·23 (6° C), 2·01 (26° C). Mean values: 26° C = 2·08; 6° C = 2·32. The elasticity has a significant *negative* temperature dependence.

3. *Hypertonic solution, R4S.* $l = 1{\cdot}03l_0$ (SREC dominant): 2·31 (7° C), 2·16 (29° C), 2·03 (7° C), 2·31 (29° C), 2·14 (8° C), 2·36 (7° C), 2·48 (26° C), 2·31 (7° C), 2·57 (26° C). Mean values: 27·5° C = 2·38 (S.D. = 0·16); 7° C = 2·23 (S.D. 0·12). The difference is not significant.

4. *Hypertonic solution, R7S.* $l = l_0$ (SREC dominant): fifty measurements of elastic modulus were made at 25° C and 8° C alternately. Mean values: 25° C = 3·67 (S.D. = 0·15); 8° C = 4·52 (S.D. = 0·16). The elasticity has a substantial *negative* temperature dependence.

Experiments 1–3 were for a toad's sartorius, the value of E being measured as the amplitude of the tension oscillation resulting from an applied sinusoidal length oscillation, amplitude 20 μ, frequency 0·2 c/s. Experiment 4 was for a frog's sartorius, using an applied amplitude of 15 μ, frequency 0·2 c/s. The elastic modulus is given as an oscilloscope deflexion and is in arbitrary units. These units are not the same for the four experiments.

TABLE 6. Effect of temperature change on resting tension

R8S		Isotonic solution	
Temperature (°C)	Tension (mg)	Temperature (°C)	Tension (mg)
17·6	1005	18·8	750
−0·8	1263	−0·1	694
18·8	977	19·4	740
−0·5	1269	−1·1	667
18·0	965	20·3	730
−0·1	1270	−0·8	667
		20·2	727

Readings were taken in the order given at each tonicity. In isotonic solution the tension is *positively*, and in hypertonic solution *negatively*, dependent on temperature. Pair frog's sartorii: l_0 = 27 mm, l = 24 mm, M = 119 mg.

trolled temperature change (Fig. 13). It was found that the tension is not determined simply by the temperature itself; the tension is considerably higher at a given temperature on the falling than on the rising phase. The meaning of this is considered later.

It has been known for many years that the resting tension of a muscle in normal solution, and at not too great a length, increases when the temperature is raised (for a review, see the Introduction of A. V. Hill's (1952) paper). The temperature coefficient has usually been expressed as $\beta = (1/P)\,(dP/dT)_l$, where P is tension, T is absolute temperature and l is length. The results of Table 6 (for the muscle in isotonic solution) gave a value of $\beta = 4{\cdot}7 \times 10^{-3}$,

which is in reasonable agreement with the mean value $\beta = (4{\cdot}06 \pm 2) \times 10^{-3}$ quoted by A. V. Hill (1952, p. 467) for the results of experiments by the earlier workers, using muscles at length l_o.

The effect of lowering the pH of the interior of the fibre. The pH inside a fibre is regulated by the internal carbon dioxide-bicarbonate buffer system, and is sensitive to the external carbon dioxide tension; it is not much affected by the external hydrogen ion concentration as such (Hill, 1955; Caldwell, 1958). The internal pH is normally about 7·0, but it can be lowered to about 6·0 by saturating the solution with a gas mixture containing a high percentage of CO_2. The

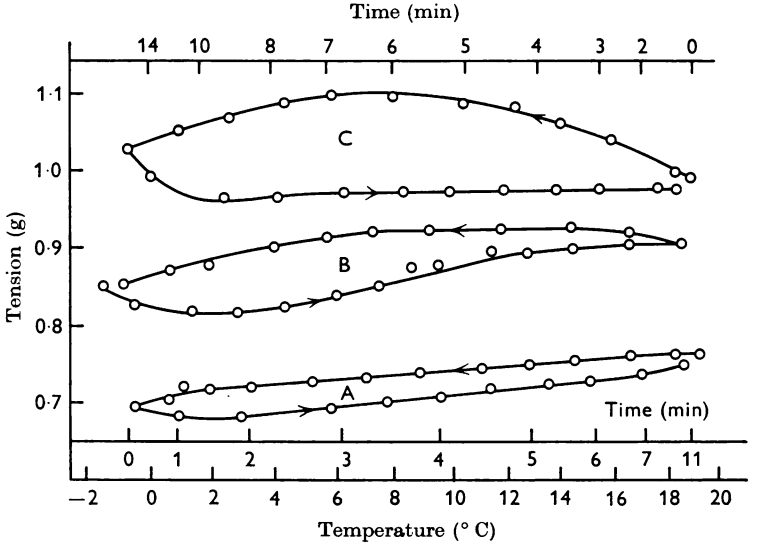

Fig. 13. Effect of changing temperature on the resting tension. Pair frog's sartorii; l_o = 28 mm, l = 24½ mm, M = 99 mg. A in R; B in R4S; C in R7S. Thermal expansion and contraction of the apparatus caused small but significant changes in the SREC tension when the muscle was maintained at 'constant' length, and to obviate this source of error a sinusoidal oscillation of 500 μ amplitude, which took the SREC beyond its elastic limit, was left running continuously. The frequency of oscillation was 0·1 c/s for A and B, and 0·05 c/s for C. The tension recorded is the mean between the maximum and minimum in the oscillation. The muscles were taken through temperature cycles, starting and finishing at the open ends of the loops. The arrows show the direction of temperature change. The upper and lower time scales show the course of the temperature change during the falling and rising phases respectively. The final temperature at the end of a traverse was approached slowly.

effect of such a reduction of internal pH on the FRT and the SREC was tested. The solution was buffered with 30 mM sodium bicarbonate, and was made hypertonic with sucrose (5 g/100 ml.) in order to give easily measurable values of the FRT and of E. These values were first measured in the Ringer/bicarbonate/sucrose solution with no CO_2 present (0° C; pH, measured, = 8·1). The change to a gas mixture containing 80 % CO_2/20 % O_2 (0° C; pH, calculated from the Henderson–Hasselbalch equation, = 5·8) had no measurable effect on the tension or on the elastic modulus (less than 5 % change). Only one experiment was done: if a large change had been found the matter would have been worth pursuing, since there

would then have been more interest attaching to it in view of the suggestion (p. 679) that electrostatic repulsive forces may produce tension in a resting muscle, because this repulsion would be expected to depend on the pH (Rome, 1967).

It is worth reporting briefly that these high concentrations of CO_2, in solution R5S, had a remarkable effect on the height and duration of the twitch. The twitch was reduced in size to 5–10 % of that recorded in the absence of CO_2 and the time course was greatly prolonged. In isotonic solution, with the same buffer, 80 % CO_2/20 % O_2 had a smaller effect, and reduced the twitch to 20–30 % of its normal height, but again the twitch was greatly extended in time. These changes were entirely reversed by removal of the CO_2. There seems to be no record in the literature of similar observations, but it should be noted that under the conditions which are generally employed, that is, in isotonic solution and at room temperature, the effect would probably be rather small, since the solubility of CO_2 is only about half as great at 20° C as it is at 0° C, and the internal pH could not be lowered so far as it was in the present experiment, even with 100 % CO_2.

The effect of stimulation

So far, the experiments have been concerned with resting muscle. The question now arises: what happens to the FRT and the tension-holding power of the SREC when a muscle is stimulated? At first it might be doubted whether any experimental evidence on this subject could be obtained. In a normal muscle the force developed during contracton is enormous compared with the FRT, or with the 'frictional resistance' of a resting muscle. In a hypertonic muscle the latter are increased in magnitude, but even though the twitch tension is reduced the mechanical strength of the inter-filamentary coupling, as indicated by the rigidity of the stimulated muscle when it is rapidly stretched (Howarth, 1958), is still enormously increased. This development of a profoundly altered mechanical state might be expected to rule out experimental observations on the FRT and the SREC.

However, it is in fact possible to make a limited study of the behaviour of the SREC (as indicated by the magnitude of the 'frictional resistance') even at the height of a tetanus. In addition, the changes which occur during the latent period after electrical stimulation can be investigated.

Experimental evidence about the FRT in a stimulated muscle is less easy to obtain; the investigation, here, has had to be restricted to making a study of the latency relaxation under different conditions, in an attempt to show that this relaxation may be due to a release of FRT. This will be considered first.

The FRT in stimulated muscle. The argument naturally centres round the question: is the latency relaxation (LR) due to a reduction of the FRT? It is admitted that no very definite conclusion is reached, and other ideas concerning the nature of the LR (for a review see Sandow, 1966) are certainly not ruled out by the present arguments, which are directed specifically to substantiating the view that the LR may be due to a reduction of the FRT.

Before describing the experiments the position will be summed up. There are three pieces of evidence in favour of the idea that the LR may be due to a reduction of the FRT. First, the LR is found to become larger *in relation to the twitch tension* if the external solution is made hypertonic. This would be expected, because hypertonicity has been shown to increase the FRT, and it is known to decrease the twitch tension (Howarth, 1958). Under normal conditions, in isotonic solution, the LR is only 1/2000 to 1/1000 of the size of the twitch (Sandow, 1945; Sandow & Kahn, 1952). In hypertonic solution the ratio increases, at first rather slowly as the solution becomes more concentrated, but above R8S the ratio rises rapidly. The LR becomes comparable in size with the positive tension and finally, at about R14S, when the positive twitch tension is practically non-existent, the latency relaxation remains on its own.

Secondly, if the LR is due to a release of resting tension generated between the two sets of filaments, it should not show a marked increase with increasing muscle length (at least over the range of lengths where the total resting tension increases rapidly with length) because such behaviour would be characteristic rather of a component which possessed longitudinal continuity throughout the fibre. In fact, as will be seen, the LR in a hypertonic muscle may be nearly the same at different lengths. (The reason why the size of the LR in a normal muscle is critically dependent on the length of the muscle is considered in the discussion.)

Thirdly, it is shown that the tension-holding ability of the SREC starts to diminish during the latency relaxation period. So, in view of the proved close association of the SREC and the FRT, this similarity of time course is a further indication that the LR is due to a reduction of the FRT.

However, from the quantitative point of view the position is not altogether satisfactory. Under normal, isotonic conditions the LR and the FRT do not differ greatly in magnitude. (The FRT is about 4 g.cm^{-2} (Table 4); the LR is about 1 g.cm^{-2}, but there would probably be a larger fall of tension if the extremely rapid positive tension development did not supervene.) But what is difficult to explain is the fact that a hypertonic solution, though it greatly increases the size of the LR in relation to the positive twitch height, is found not to increase the *absolute magnitude* of the LR. The FRT increases in a hypertonic solution, so it is puzzling to find that the LR does not do so too. However, this discrepancy is not thought to represent a serious objection to the idea that the LR is due to a reduction of FRT, because experiments with the SREC, where the interpretation is more certain, show that here, too, the tension produced by a slow continuing stretch is only slightly reduced during the latency relaxation period (though the suppression is practically complete at the height of tetanus). So, by arguing again on the basis of the known associa-

tion of the SREC and the FRT, the failure of the LR to show an increase with hypertonicity is not inconsistent with the present scheme. An explanation for the discrepancy is suggested later.

An account of the experiments now follows.

Technical considerations. It was not possible to record the LR in isotonic solution, or in the weaker hypertonic solutions. The photoelectric recorder was too 'noisy' for the frequency response required. And, also, the method of stimulation was not suitable. The velocity of conduction of the action potential in frog's muscle fibres at 0° C is about 1·0 m.sec^{-1} (estimated from data given by Buchthal & Engbaek, 1963). In a muscle 25 mm long the time taken to travel the length of the muscle is 25 msec. The LR of a frog's muscle in isotonic solution lasts only 10 msec at 0° C (Abbott & Ritchie, 1951). Multi-point stimulation is necessary to record the full effect (Sandow, 1945; Sandow & Kahn, 1952), and end-to-end stimulation, as used here, would be expected to show only a greatly reduced LR. Similar objections to the methods do not apply in the experiments with muscles in the stronger hypertonic solutions because, as will be seen, the LR is then greatly extended in duration; so high-frequency tension recording is not required, and the conduction time is not important.

The latency relaxation in hypertonic solution. Single shocks. The LR/twitch tension ratio is 1/2000–1/1000 in isotonic solution. The absolute magnitude of the LR does not change much when the solution is made hypertonic, but the twitch height diminishes progressively as the tonicity is raised. The twitch height in solution R7S is about half its normal value: two experiments showed LR/twitch ratios of 1/590 and 1/760. In stronger solutions the twitch falls off more rapidly, and the ratio increases to 1/10–1/4 in R11S (Figs. 14, 15). In solution R13S the positive phase may be about equal to the LR.

At about R14S the interesting state is reached where the positive tension development is probably totally suppressed. (A succession of shocks will cause a gradual production of tension in solutions as strong as R17S.) The tension does not rise above the base line, but it returns towards it (Figs. 14, 15), and this recovery of tension lost during the downstroke is thought to represent the reversal of the LR process. An alternative explanation would attribute such tension recovery as remains to a remnant of the normal positive process, but this would involve the assumption that the recovery of the LR was indefinitely delayed. This assumption would be hard to accept; but the first explanation is preferred in any case, because it has been shown that the rise from the bottom of the LR cannot be abolished by a further increase of concentration. At, or above, R16S the LR becomes smaller and finally vanishes altogether, but the rise from the bottom of the LR, as it gets smaller, is never lost.

One remarkable feature of the LR in hypertonic solution is the extension of the time scale of events. In normal muscle at 0° C (Abbott & Ritchie, 1951) the LR starts at about 8 msec, and the negative peak is at 15 msec. With increasing hypertonicity the time scale is progressively drawn out,

and with a frog's muscle in, for instance, R12½S at 0° C (Fig. 15*C*) the LR starts at about 50 msec, and the negative peak is at 150 msec; this is a tenfold extension of the time scale. Under normal conditions a toad's muscle is about half as fast as a frog's, and the same difference is seen in hypertonic solution (Fig. 14).

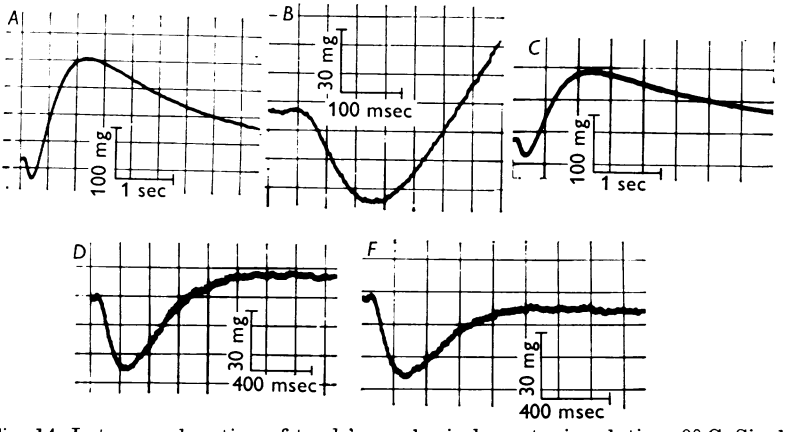

Fig. 14. Latency relaxation of toads' muscles in hypertonic solution, 0° C. Single shock at beginning of trace. Two experiments: *A*, *B*, and *F* are the records for a pair of sartorii with l_0 = 26 mm, l = 24½ mm, M = 66 mg. *C* and *D* are for another pair of sartorii, with l_0 = 27 mm, l = 23½ mm, M = 75 mg. For *A* and *B* the solution was R11S; for *F*, solution R14S; for *C*, solution R13S; for *D*, solution R15S.

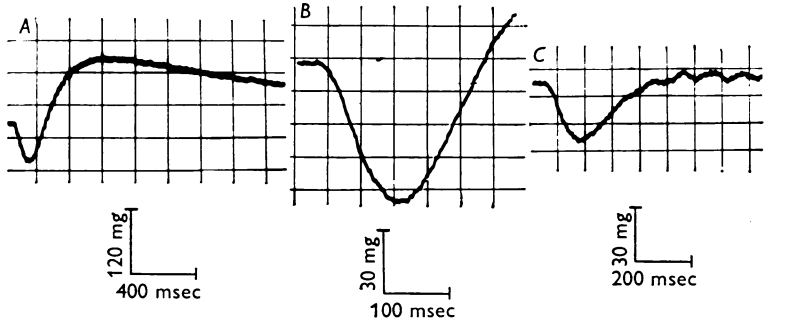

Fig. 15. Latency relaxation of frog's muscles in hypertonic solution, 0° C. Single shock at beginning of trace. Pair sartorii, l_0 = 30 mm, l = 27½ mm, M = 167 mg. For *A* and *B* the solution was R11S. For *C*, the solution was R12½S.

Some variability was found in the effect of a given degree of hypertonicity, and it was often necessary to make fine adjustments of the sucrose concentration (by 1 or 2 %) to produce the required LR/twitch ratio. It was also invariably found that the ratio increased very slowly with time in the same solution. Hodgkin & Horowicz (1957) observed a similar protracted decline of the twitch tension of single fibres following the initial rapid change after immersion in a hypertonic solution.

The effect of repeated stimulation. Under normal conditions the twitch is so large that it would be difficult to test the ability to elicit a second LR during the progress of the twitch. But in a sufficiently hypertonic solution the positive phase is so much suppressed that such a test can be made.

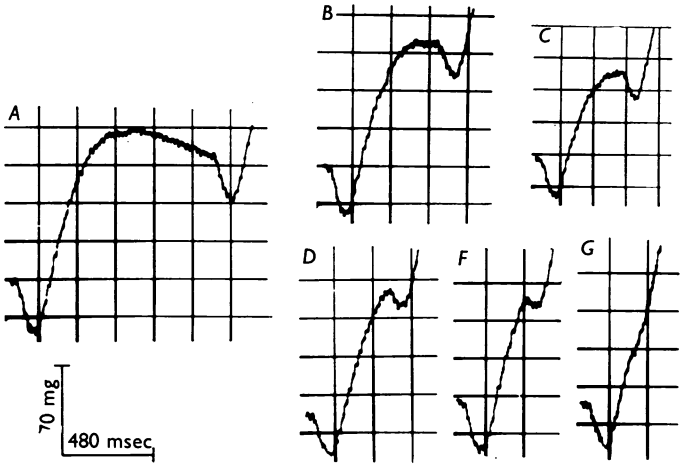

Fig. 16. Experiment to show that a second shock applied during a twitch will again produce relaxation. Solution, R11S. Pair frog's sartorii, l_o = 25 mm, l = 21½ mm, M = 60 mg, 0° C. Time intervals between shocks: *A*, 1000 msec; *B*, 550 msec; *C*, 500 msec; *D*, 380 msec; *F*, 300 msec; *G*, 220 msec.

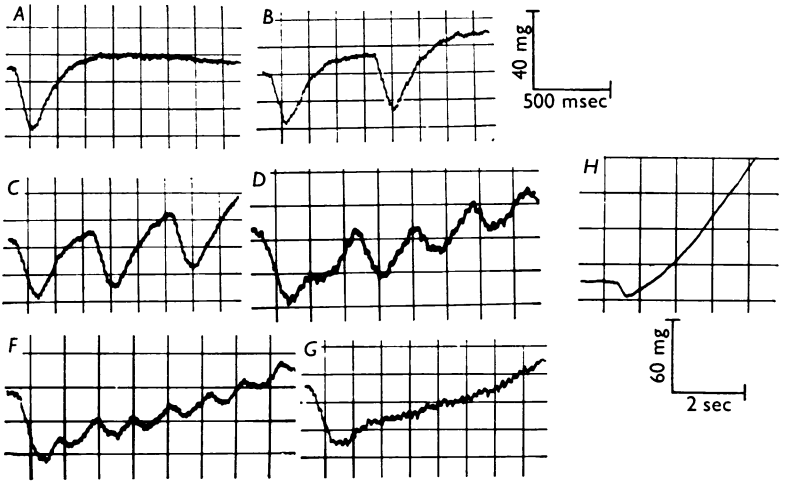

Fig. 17. Latency relaxation with repetitive stimulation. Strongly hypertonic solution. Pair frog's sartorii, l_o = 25 mm, l = 21½ mm, M = 60 mg, 0° C. For *A* to *G* the solution was R13S. Time intervals between shocks: *A*, single shock only; *B*, 800 msec; *C*, 600 msec; *D*, 350 msec; *F*, 220 msec; *G*, 140 msec. The record *H*, using solution R15S shows, on a slow time base, how the positive tension slowly builds up with repetitive stimulation, with shocks spaced at 140 msec. The tension and time scales for records *A* to *G* are given at the top, and the scales for *H* below.

The experiments of Figs. 16 and 17 show that a second shock, or a succession of shocks, is capable of producing a further response during the contraction. A second LR is of normal size only when it occurs at, or after, the peak of the twitch. When the time interval between the shocks is reduced so that the second LR occurs before the peak of the first twitch it is reduced in size, and this reduction becomes more marked as the time interval becomes shorter. The experiment of Fig. 18 shows that a small effect is still seen even when the second relaxation starts at the lowest point of the first. The relaxation phase, and the delay in returning to the base-line, can be prolonged considerably by a succession of shocks suitably spaced (Fig. 19). If the interval is reduced sufficiently the second shock contributes nothing, either to the relaxation or to the positive tension (Fig. 18*A*).

The following tentative conclusions may be drawn. First, the recovery of the ability to elicit a full-size LR at the peak of a preceding twitch suggests that the process responsible for the first LR has completely reversed by that time. This is consistent with what has been said, above, about the meaning of the records (Figs. 14, 15) where the positive tension phase is thought to have been completely suppressed. Secondly, the failure to elicit any relaxation whatsoever by means of a second shock if the time interval after the first is short (but when it is still much longer than the refractory period for membrane excitation), indicates that a single shock, by itself, releases all the tension that is available. The difficulty here, however, is that, if it is to be accepted that the LR is due to a release of FRT, it is necessary to account for the fact that the LR is, in magnitude, only a small fraction of the FRT which is present in a strongly hypertonic muscle (some values are given later). The most likely explanation is that the internal 'mechanical' events cannot be manifest externally in the time available. A total collapse of the FRT would be correctly recorded externally only if the filaments were able to slide sufficiently, in the time available before the reversal of the process sets in (at the lowest point of the LR), to release the appropriate amount of tension in the series elastic elements. The force required to move the filaments is almost independent of velocity under the conditions where the SREC was investigated (p. 647), and *viscous* forces have therefore not, as yet, been mentioned. But in a strongly hypertonic muscle viscosity may be an important factor. It could possibly cause so much delay in the external appearance of internal mechanical events that it would lead to the present large difference between the LR and the FRT. (It may be that the effect of extreme hypertonicity in reducing the positive phase of the twitch is due to the same cause. There is no evidence of a failure of the activation process after a single shock, as indicated by thermal changes (A. V. Hill, 1958), even though no tension is produced.)

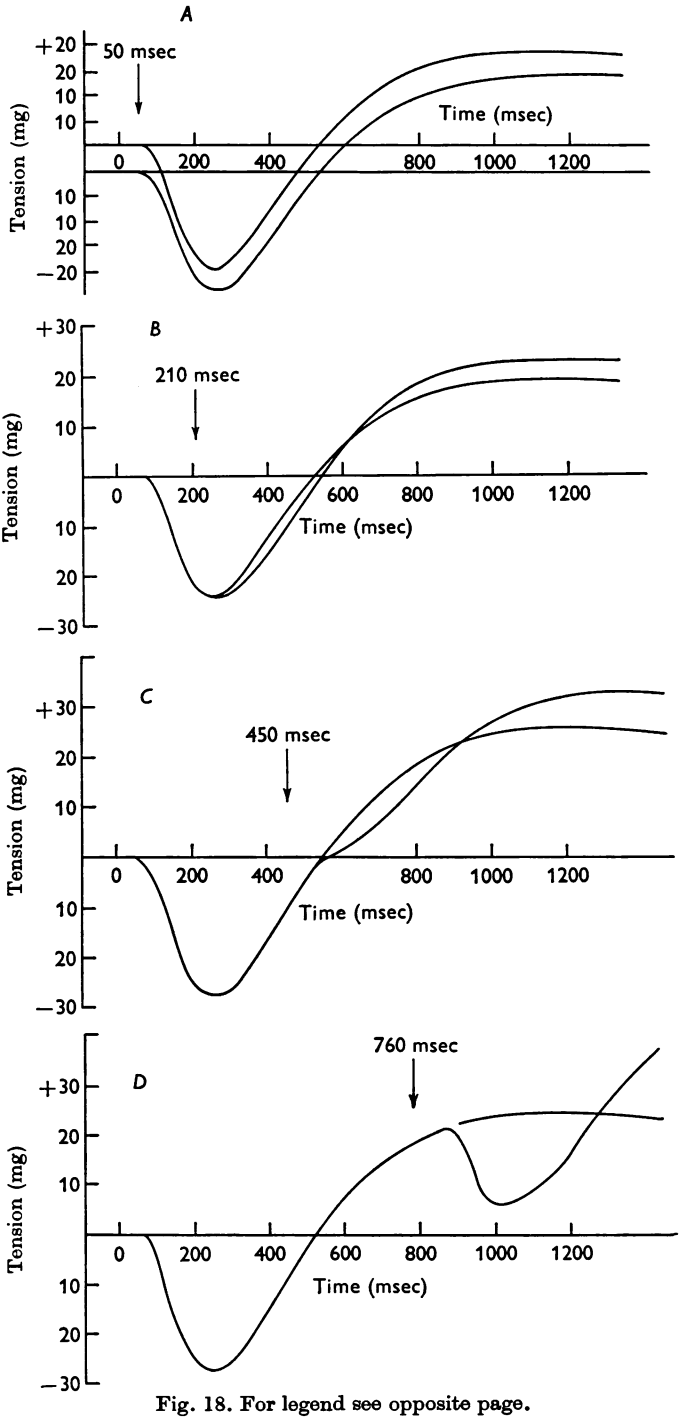

Fig. 18. For legend see opposite page.

Latency relaxation at different lengths. Under normal, isotonic, conditions the size of the LR is critically dependent on the length of the muscle. It is at its full size in a muscle which has been stretched several millimetres beyond l_0. At l_0 it is much smaller, and when the length is only a millimetre or two less than l_0 it vanishes altogether (Sandow, 1945; Sandow & Kahn, 1952; Abbott & Ritchie, 1951). In hypertonic solution the behaviour is entirely different. In R8S, or in stronger solutions, the LR is practically independent of the length of the muscle. It became clear, from many

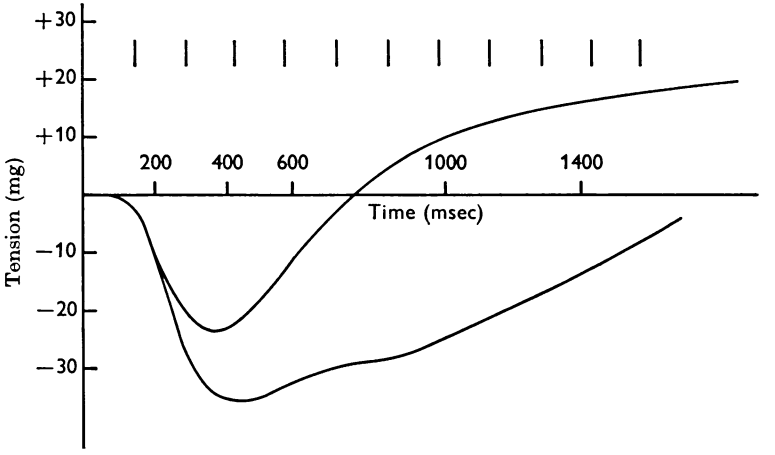

Fig. 19. The tension change due to stimulation with a succession of shocks spaced at 140 msec (lower curve), compared with the effect of a single shock (upper). Plotted reproductions of photographic oscilloscope records. Pair frog's sartorii, l_0 = 25 mm, l = 21½ mm, M = 60 mg, 0° C, solution R15S. The vertical lines indicate the times of the shocks for the lower record.

unrecorded observations, that the size of the LR over the range of sarcomere lengths from 2·0 to 2·6 μ was so nearly constant that a large number of photographic records would have to be made to establish whether there really is any definite correlation with length. Such an analysis was not undertaken, but it is unlikely that a change of more than 10% would be found over that range.

In less strongly hypertonic solutions, the dependence on length, which is characteristic of the isotonic muscle, would presumably start to appear.

Legend to Fig. 18.

Fig. 18 *A–D*. The tension change due to pairs of shocks spaced at different intervals of time. Plotted reproductions of photographic oscilloscope records. Pair toad's sartorii, l_0 = 27 mm, l = 23 mm, M = 60 mg, 0° C. Solution R13S. The first shock was given at time zero and the second at the time indicated by the arrow. In *A*, the upper record is for the doubly stimulated muscle; the records here are practically identical, and the base line has therefore been duplicated to separate the curves.

It is under these conditions that the FRT increases when a muscle is stretched (Figs. 9, 10). Experiments were not made to test whether the FRT shows less variation with length in the more strongly hypertonic solutions, as would be expected if the size of the LR is a constant fraction of the FRT.

The size of the LR at different tonicities. For reasons which have been given the LR could not be recorded in isotonic, or in the weaker hypertonic solutions, so it was not possible to make a study of the size of the LR through the whole range of tonicities. It became clear, however, that the LR does not increase in magnitude with increasing tonicity in the same way that the FRT does. A few figures illustrate this point. In isotonic solution it may be assumed that the LR is about 1 g.cm^{-2}. The LR in the experiment of Fig. 14*C* (solution R13S) was 1·0 g.cm^{-2}; the same muscle in R15S gave 1·3 g.cm^{-2}. In Fig. 14*B* (R11S) the LR was 1·7 g.cm^{-2}; the same muscle in R14S gave 1·3 g.cm^{-2}. In Fig. 15*B* (R11S) the LR was 1·1 g.cm^{-2}; the same muscle in R12½S gave 0·5 g.cm^{-2}. On the other hand, the FRT, equal to about 4·0 g.cm^{-2} in isotonic solution, increases at least ten times in a muscle which has been bathed in solution R11S (in Fig. 11 the FRT rose to 50 g.cm^{-2}), or to even higher values in more concentrated solutions.

The SREC in stimulated muscle. The absence of a 'frictional resistance' to a slow length change during a maintained contraction. The working hypothesis (see Summary) suggests that the short-range elastic properties of a resting muscle, and the ability to show a 'frictional resistance' to a slow length change, are due to the presence of stable, long-lived cross-bridge linkages. The elastic modulus and the magnitude of the 'frictional resistance' are nearly unchanged down to extremely low velocities (Figs. 6, 7) and this shows that the average 'lifetime' of a formed contact, which has to be long enough to allow the SREC to be strained out to its 'elastic limit' (about 0·2% of l_0), is probably many seconds. A contracting muscle, on the other hand, is capable, when lightly loaded, of shortening by 0·2% of l_0 in a few milliseconds. The average 'lifetime' of a cross-bridge connexion in a contracting muscle is therefore probably several orders of magnitude shorter than it is in the resting muscle. If this is the correct interpretation, and the hypothesis is tenable (and, at rest, the structural elements involved really are the cross-bridges), then the 'frictional resistance' to a slow continuing length change should be found to vanish when the muscle is made to contract.

In making experiments to test this point it is necessary to avoid confusion from the very large tension changes which occur when a contracting muscle is forcibly lengthened, or is allowed to shorten, at constant velocity, even when this velocity is quite low. Fortunately, the 'frictional resistance'

of a resting muscle remains nearly unchanged at extremely low velocities, where the tension changes caused by lengthening or release of a contracting muscle become small compared with the 'frictional resistance'. Therefore, the experiment is done by comparing the force–velocity relation of a contracting muscle at these extremely low velocities, with the 'frictional resistance' shown by the same muscle, when unstimulated, over the same range of velocities.

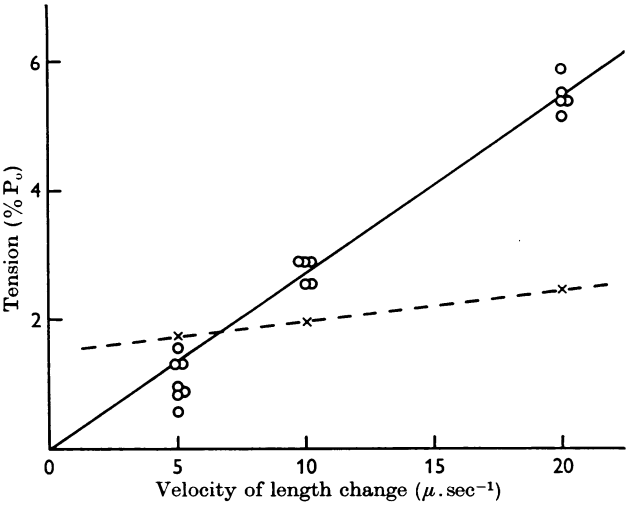

Fig. 20. The effect of a continuing length change at constant velocity on the tension in a contraction maintained by repetitive stimulation. Single frog's sartorius, l_o = 27 mm, l = 27 mm, M = 53 mg, 7° C, solution R6S. The maximal tetanic tension was 1·64 kg.cm^{-2}. The continuous line (○) shows the difference between the tensions developed in a contraction during lengthening and a contraction during shortening. The broken line (×) shows 2 × the amount of 'frictional resistance' developed during a continuing lengthening of the same muscle at rest.

The results of two experiments are shown in Figs. 20 and 21. The muscles were bathed in hypertonic solutions in order to increase the 'frictional resistance' at rest, but these solutions were not so strong as to cause too great a suppression of the contractility. The length change was started while the muscle was at rest, and the stimulation current was switched on when the applied length change was about 100 μ (this length change would have produced the full 'frictional resistance'). The tension difference in the contracting muscles, as between lengthening and shortening, has to be compared with twice the amount of 'frictional resistance' developed by the same muscle at rest, and the results are plotted accordingly.

The results (Figs. 20, 21) show clearly that the 'frictional resistance' of the resting muscle is no longer present (or at least it is much reduced) in the contracting muscle. The line showing the difference between the

tensions during lengthening and during shortening of the contracting muscle is nearly linear, and passes through the origin, for velocities below 10 μ.sec^{-1} where, in a resting muscle, the 'frictional resistance' is not only comparable with this difference, but remains nearly independent of the velocity. What is more, at 5 μ.sec^{-1} and below, the tension increment during contraction is actually less than the 'frictional resistance', and in solution R8S (Fig. 21) it is much less.

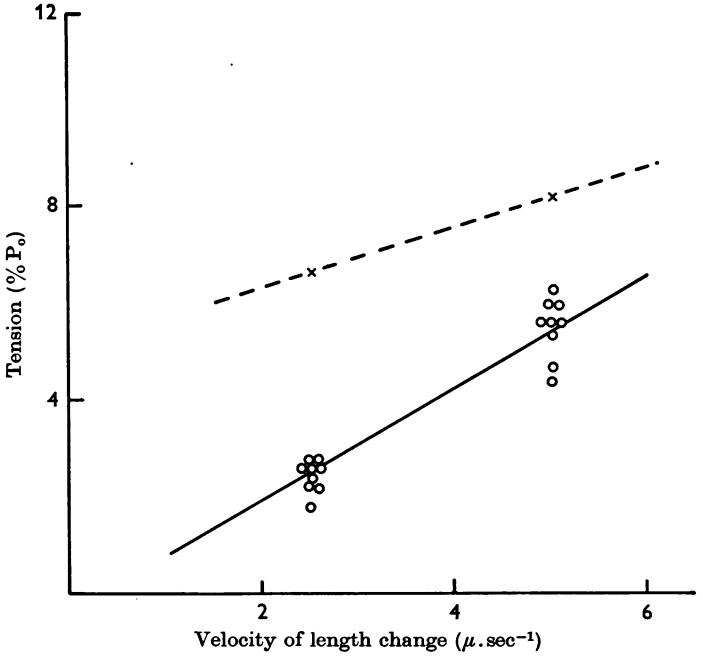

Fig. 21. An experiment similar to that shown in Fig. 20. Single frog's sartorius, l_o = 27 mm, l = 28 mm, M = 52 mg, 7° C, solution R8S. The maximal tetanic tension was 0·93 kg.cm^{-2}.

The SREC during the latent period. While the experiments on the latency relaxation of a hypertonic muscle were being made it was noticed that a small stretch, completed a few seconds before the stimulus was given, caused a considerable increase in the size of the latency relaxation. At first it was thought that this might be due merely to a reduction in the compliancy of the system, brought about by the temporary increase in the resting tension. But a calculation showed that if the latency relaxation is due to *a fixed length change* in the contractile system (or its series elastic elements) the increase of relaxation caused by the stretch was much greater than could be explained that way. It follows, therefore, that when the tension is raised, by stretching the SREC, the contractile system

elongates by a greater amount, during the latency relaxation, than it does under steady-state conditions. This could be explained in two different ways. First, there is the possibility that the release of tension during the normal LR process is accelerated by imposing an additional tension on the system. Alternatively, the increase in the amount of relaxation could be due to a release of some of the tension still held by the SREC after the preliminary stretch. It was not difficult to decide between these two

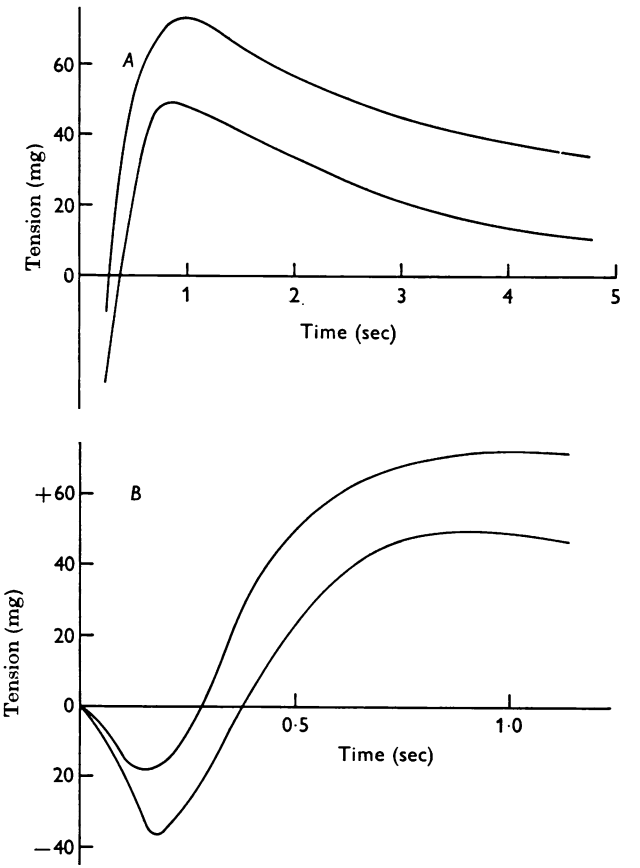

Fig. 22 *A*, *B*. The tension change due to single-shock stimulation applied during a slow imposed lengthening of the muscle (lower record), compared with the change during a slow imposed shortening (upper record). Pair toad's sartorii, l_0 = 27 mm, l = 29 mm, M = 60 mg, 0° C. Hypertonic solution, R12S. The elongation, or shortening, at velocity 2·5 μ.sec^{-1}, was started 25 sec before the stimulus was applied. With this strength of solution the tension during the continuing length change before stimulation failed to come quite to a steady value. The actual records therefore had rising, or falling, 'base lines', to which the effects of stimulation had to be referred, and correction has been made for this. The time scale has its zero at the point of the first detectable relaxation, and not at the time of stimulation.

possibilities. The method depends on the following argument. The process producing the latency relaxation is probably reversed quite rapidly; there is evidence to suggest (p. 667) that the reversal is complete by the time the twitch reaches its peak. On the other hand, when the SREC is stretched by applying a continuing elongation to the muscle, any loss of tension due to a collapse of the SREC will be regained only after a period of time which is sufficient to re-stretch the SREC to give its full initial tension. If the speed of stretch is sufficiently low, this latter time interval, required to re-stretch the SREC, can be made much longer than the time taken for the recovery of the LR process. Therefore, if the extra latency relaxation caused by stretching the muscle is due to a collapse of the SREC, and the speed of stretch is sufficiently low, there should be a continuing tension deficit (below the value recorded with an unstretched muscle) for a prolonged period of time, continuing well beyond the peak of the twitch. An experiment to test this point is shown in Fig. 22. Here a comparison was made of the tension record of a twitch during a slow lengthening of a muscle, at a velocity of $2{\cdot}5\,\mu.\mathrm{sec}^{-1}$, compared with that during a slow shortening at the same velocity: this reversal procedure was employed to double the effect. The muscle was equilibrated with strongly hypertonic solution, in order to suppress most of the positive tension development, and the total duration of the twitch was too great to allow the whole of the relaxation phase to be followed with sufficient accuracy. However, it is clear enough what is happening. The extra relaxation seen when the SREC is stretched is not rapidly recovered. Even at 5 sec there is apparently no appreciable recovery. The length change required to produce the full SREC tension in this muscle was about 90 μ, and at a velocity of $2{\cdot}5\,\mu.\mathrm{sec}^{-1}$ the re-stretching of the collapsed SREC would take 36 sec. Presumably, after the lapse of this period of time the tension difference as between the records for stretch and for shortening must have disappeared.

The conclusion is that the increase in size of the latency relaxation caused by stretching a muscle is due to a reduction of the tension generated in the SREC by the stretch.

A similar experiment, but at a lower tonicity, is shown in Fig. 23. Here the positive tension became so large that the analysis had to be confined to the earliest stages. The indications are, however, that the result is essentially the same as it is in the strongly hypertonic solution.

The amount of SREC tension released during the latent period. In the experiment of Fig. 22 the tension released during the LR period amounted to 20 mg, or about $1{\cdot}0\,\mathrm{g.cm}^{-2}$. This is only a very small fraction of the total tension held by the SREC. The latter was not measured in that experiment, but the elastic modulus was probably about $40\,\mathrm{kg.cm}^{-2}$ (Fig. 4) and for an 'elastic limit' equal to about 0·2 % of l_0, the tension

held ('frictional resistance') would be 80 g.cm^{-2}. The *recorded* loss of SREC tension, during the LR period, was only 1–2 % of the total. All the other experiments, when strongly hypertonic solutions were used, showed a difference of the same order. Yet in a full tetanic contraction it has been shown that practically all the 'frictional resistance' to a slow length change is lost.

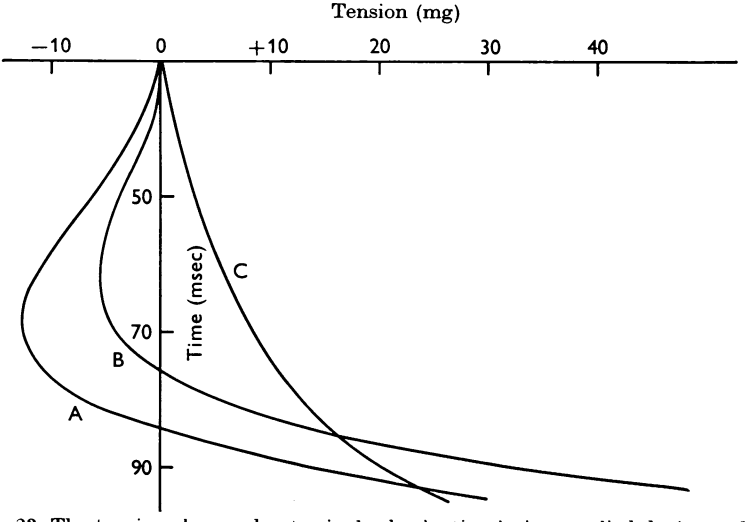

Fig. 23. The tension change due to single-shock stimulation applied during a slow imposed lengthening of the muscle (*A*), compared with the change during a slow imposed shortening (*B*). Each curve is the mean of eight records. The difference between *A* and *B* is shown at *C*. Pair toad's sartorii, l_0 = 25 mm, l = 27 mm, M = 57 mg, 0° C. Hypertonic solution R7S. The elongation or shortening, at velocity 36 μ.sec^{-1}, was started 3 sec before the stimulus was applied. The amplifier was condenser-coupled, with time constant 0·33 sec, and the base line was therefore level; no correction, as in Fig. 22, was necessary. In this case the time scale has its zero at the time of stimulation, and not at the point of the first detectable relaxation as in Fig. 22.

If the hypothesis is correct, these findings need some explanation. It is suggested that the large 'discrepancy' is probably caused by the fact that 'internal' mechanical events of short duration cannot be recorded externally in the time available. The argument is similar to that put forward (p. 667) in connexion with the FRT and the latency relaxation.

The recovery of the tension-holding power of the SREC. It is seen (Fig. 22) that the loss of tension attributable to a reduction of the tension-holding power of the SREC during a slow stretch does not continue after the peak of the positive tension has been reached. It may therefore be concluded that the SREC regains its ability to hold a 'frictional resistance' against a slow length change by the time this stage is reached, and that the suppression is not prolonged during the relaxation phase. It has been seen that

the ability to show a second full-size latency relaxation is recovered at about the same time. This is further evidence for the view that the FRT and the SREC are related in the way suggested in the working hypothesis.

The twitch tension at different lengths in hypertonic muscle. Though there may be no immediate relevance to the present subject, the opportunity is taken to put on record certain observations concerning the twitch in hypertonic solution. With a normal muscle the tension in an isometric tetanus is proportional to the length of overlap between the two sets of filaments (Gordon, Huxley & Julian, 1966). In hypertonic muscle the tetanus tension is lower (Howarth, 1958), but it has been shown, in the present series of experiments, that this tension is still almost exactly proportional to the amount of overlap at different lengths. But with the twitch the result is different. In normal muscle the twitch tension, like the tetanus tension, is very nearly proportional to the length of the overlap region. But with a muscle in hypertonic solution of sufficient strength a remarkable effect appears: the twitch tension per unit length of overlap is now greatly reduced by lengthening the muscle. This is shown by the following figures for a pair of frog's sartorius muscles (l_0 = 25 mm, M = 94 mg, 0° C.)

	Twitch tension (g)				
Length (mm)	Normal Ringer	R4S	% of normal	R8S	% of normal
23	67·3	27·8	41·4	0·67	0·99
25	60·2	24·5	40·6	0·25	0·42
27	51·8	19·8	38·2	0·12	0·23
29	38·6	14·1	36·5	0·05	0·13

The effect in R4S, where the twitch tension is still 40 % of its normal value, is small. But in R8S, where the twitch is only 1 %, or less, of normal, the tension per unit length of overlap is much less at the greater than at the smaller lengths.

Calculation shows that, as with the SREC (p. 652) and the FRT, the effect of hypertonicity in reducing twitch tension is mainly due to an increase in concentration of the sarcoplasm, but the results just given show that a reduction of the gap between the filaments, by working at a greater length, may also have an inhibitory effect on tension production.

DISCUSSION

The assumption is made, in the working hypothesis (described in the Summary), that the tension-generating mechanism in a resting muscle is identical with that responsible for contraction. It involves the two sorts of filaments, linked by the cross-bridges. This scheme has the merit of not having to postulate that there is a dual mechanism. It also obviates the difficulty, which would arise if a dual mechanism were proposed, of finding a reason to explain why stimulation has the effects it does. It has been suggested that the mechanism responsible for the various resting tension effects is, in some sense, 'switched off' as the result of stimulation. But there is no 'mechanical' necessity for such a suppression. The 'frictional resistance' of an isotonic resting muscle, during a length change, is far too small to have any effect on the performance of a contracting muscle. On the other hand, in accepting the idea that only a single mechanism is involved, it still remains necessary to explain why the effects observed in

the resting muscle are suppressed as the result of stimulation. Two questions require answering. First, why is a stimulated muscle no longer capable of showing a 'frictional resistance' to a continuing slow length change; secondly (if it is accepted that the latency relaxation is due to a reduction of the FRT), why does the tension of a muscle *decrease* before the main contraction sets in? The explanation may be as follows. In describing the working hypothesis, and again on p. 670, it was pointed out that, in a resting muscle, the average 'lifetime' of the cross-connexions must be supposed to be long enough to allow a measurable tension to be built up in the cross-bridges when the filaments are made to slide past one another at a low velocity. A display of 'frictional resistance', which is nearly independent of the velocity of the relative motion of the sliding parts, depends on the existence of a type of cross-contact which breaks open only when the force reaches a critical limit. The frequency of breakage of an unstressed, or lightly stressed, connexion must be low.

If this is the correct interpretation, it follows that the suppression of the ability of a stimulated muscle to show a similar 'frictional resistance' is attributable to a large increase in the frequency of the breakage of the cross-links, even when they are not under mechanical stress.

It should be pointed out, incidentally, that another essential requirement of any sliding system, if it is to show a frictional resistance, is that a connexion which is broken by force shall be rapidly replaced by another, after a small amount of 'slip' has occurred: in the muscle this means, presumably, that the forcible breakage of a cross-connexion does not result in the abolition of the 'active state' of the cross-bridge in question, but it retains its potentiality and can immediately re-form another connexion further along the actin filament.

As to the meaning of the latency relaxation the argument could be as follows. It can be supposed (as in A. F. Huxley's (1957) hypothesis to explain the mechanism of contraction), that each cross-bridge goes through cycles of activity in which it combines with the actin filament by one reaction and is separated from it by another. The reactions must be thought of as being independent of one another, and there is no need to assume that the changes in their respective rates, which accompany the transition from rest to activity, necessarily follow exactly the same time courses. It has been argued, above, that the rate of the reaction which separates the cross-bridges from the actin filaments is probably increased by stimulation. If the change of rate of this reaction starts a few milliseconds earlier than the rate change of the reaction which forms the cross-connexions, the effect would be as observed, namely a fall of tension (latency relaxation) before the rise.

Previous ideas concerning the nature of the latency relaxation. Sandow

(1966) has summarized the present position and there is no need for a further general review. It has recently been suggested (Sandow, 1966) that the latency relaxation may be due to a release of tension in the membranes of the sarcoplasmic reticulum as the result of osmotic shrinkage of the reticulum when Ca^{2+} is released from the terminal sacs following the arrival of the excitatory impulse. This idea is based largely on a detailed analysis of the dependence of the time course of the latency relaxation on the length of the muscle. Sandow (1966) emphasizes the importance of the time interval between the onset of the latency relaxation and the moment when the relaxation reverses to give the positive contractile phase. This time interval increases when the length of the muscle is increased. Quantitatively, the change is of the same order of magnitude as the estimated additional time required for the diffusion of Ca^{2+} from the terminal sacs (near the Z line) to the more distant filament-overlap region of the stretched muscle. It might be thought that further information on this point could be obtained with a hypertonic muscle, by making a detailed analysis of the time course of the latency relaxation at different lengths—that is, by repeating what has been done using normal muscle, but with the advantage, in the hypertonic muscle, of the ability to elicit the latency relaxation at the shorter sarcomere lengths. However, further consideration of the time scales reveals a serious difficulty. The time required for diffusion of Ca^{2+} over a distance equal, say, to the length of one half sarcomere is of the order of only one millisecond (Sandow, 1966). But in a hypertonic muscle the time course of the latency relaxation becomes so prolonged (Fig. 15) that the time interval between the onset and its reversal is about 100 msec. A change in this time interval, with stretch, amounting to only about one per cent of the whole, would be very difficult to detect. It is unlikely, therefore, that Sandow's hypothesis could be tested in a hypertonic muscle, by an examination of the length dependence of the time course of the latency relaxation.

Turning to the older ideas, some remarks should be made about the possibility, which was favoured for some time, that the latency relaxation is due to a length change in one of the filamentary components of the muscle. This has recently been discussed by H. E. Huxley & Brown (1967). It was suggested, both by A. F. Huxley (1957) and by H. E. Huxley (1960) that the latency relaxation could be explained if it was assumed that the resting tension was borne by the S filaments, earlier postulated (Huxley & Hanson, 1954; Hanson & Huxley, 1955) as joining together the ends of the actin filaments across the H gap, and that the actin filaments increase in length by a few per cent when the muscle is activated, thereby releasing the tension in the S filaments. However, it is now known (Huxley & Brown, 1967; Elliott, Lowy & Millman, 1967) that the actin filaments do not in

fact elongate by anything like the required amount when the muscle is activated. This earlier idea should be abandoned on those grounds alone. The results of the present paper are also opposed to that explanation, because it has been shown that the latency relaxation is still present in a hypertonic muscle when it has been allowed to shorten so far that there is no longer any gap between the ends of the actin filaments.

Reference should be made to a suggestion by Huxley & Brown (1967). There is evidence for the existence of electrostatic repulsive forces acting transversely between the filaments (Elliott, 1967; Rome, 1967). The constant-volume behaviour of the filament lattice during stretch (Huxley, 1953; Elliott, Lowy & Worthington, 1963) requires that the filaments move nearer together when a relaxed muscle is stretched, and if there is an electrostatic repulsion between them the force necessary to move them together must appear as a resting tension. If the electrostatic repulsion decreases as the result of activation, as might possibly be the case, then the resting tension would fall to give a latency relaxation. There are no reliable data, at present, for assessing such an hypothesis in quantitative terms.

The absence of the latency relaxation at shorter muscle lengths in isotonic solution. In normal Ringer solution a latency relaxation cannot be elicited at the shorter muscle lengths. This behaviour is in marked contrast with what happens in the more strongly hypertonic solutions, where the latency relaxation may be practically independent of the length of the muscle. Actually the latency relaxation might be expected to show some increase with elongation of the muscle, for the following reasons. First, the FRT has been shown to increase when the muscle is stretched; if it is accepted that a reduction of the FRT is the cause of the latency relaxation, then the latter should increase with elongation. Secondly, the positive twitch tension decreases with elongation, and the consequent slower onset of a positive phase should allow more relaxation to be seen. Thirdly, the compliance of the system decreases at the higher resting tension of the elongated muscle; this would increase the tension recorded for a given length change in the contractile system. The compliance factor alone could not be the cause of the variation of the latency relaxation with length, because although a change of compliance would alter the rate of fall of tension, it would equally affect the rate of rise of the positive tension, and the time at which the tension curve re-crossed the base line ought to remain unchanged. In fact, the duration of the period in which the tension is below the resting value increases when the muscle is stretched (Sandow, 1944; Abbott & Ritchie, 1951). However, it could be supposed that in an isotonic muscle all three of the above factors together combine to give the observed critical dependence on length. To make this acceptable, and to

explain why a hypertonic muscle does not show a similar effect, it would probably be necessary to assume that the FRT under isotonic conditions increases much more rapidly with elongation than it appears to do in hypertonic muscle. Unfortunately, in isotonic muscle the FRT is so small compared with the other forms of resting tension, that its length dependence cannot be ascertained.

The long-range elastic properties of muscle. A. V. Hill (1952) has shown that a resting muscle possesses two quite distinct types of elasticity. At shorter lengths only one type, apparently due to a 'rubber-like' component, is involved; at greater lengths another component, with 'normal' elasticity, also comes in. The 'rubber-like' component is not at all similar in its behaviour to the SREC, because the former shows long-range elastic effects (it has no 'elastic limit'), and there is no spontaneous relaxation of the tension developed in it. But it is suggested that the 'rubber-like' component may perhaps be related to the FRT. The latter has been shown to increase with muscle length, so the mechanism responsible for it has, in effect, long-range elastic properties. Further, the 'rubber-like' elasticity and the FRT are both positively temperature-dependent when the muscle is in isotonic solution.

The term 'rubber-like' may be rather misleading, because it is not certain that this type of elasticity is thermokinetic in origin and dependent on a change of entropy with deformation. The alternative explanation is that chemical reactions are involved in producing this form of resting tension. There is some reason to prefer this explanation. The positive heat which acted as A. V. Hill's (1952) measure of the 'rubber-like' elastic effects following a sudden stretch, was not instantaneous in its appearance, but its production was spread over a period of about one second following the applied length change. This behaviour is not that of a simple thermokinetic elastic system. It is characteristic, rather, of a system where a new chemical steady state, involving changed concentrations of reactants, has to be established over a finite period of time when the length is suddenly changed. With regard to the FRT, there is also reason for suspecting that chemical reactions are involved. It has been shown that the resting tension at a particular length is not determined only by the instantaneous value of the temperature, but is affected also by the manner in which the temperature is changing. The 'hysteresis' in the temperature cycle (Fig. 13) could be attributed to a time lag in the establishment of the chemical steady state appropriate to the temperature in question.

Resting metabolism and stretch. It has been known for many years that a frog's muscle shows an increase of resting metabolism when it is stretched. This 'stretch response' (SR) has recently been studied, anew, by Clinch (1965, 1968). The FRT has also been shown to increase when the muscle

is stretched; if this form of tension is maintained by a metabolic process an increase in its rate, with stretch, might be the origin of the SR. The SR is found only when a muscle is stretched about 30 % beyond the normal length in the body, and it is possible to explain this by supposing that, in a normal muscle, the FRT increases steeply with length in the highly stretched muscle. It has already been suggested, above, that a critical length dependence of this sort may be the cause of the large change in size of the latency relaxation of a normal muscle when it is stretched beyond l_0. The SR is decreased by hypertonicity (Clinch, 1965); this, also, is consistent with the present interpretation, because it has been seen that the latency relaxation (which, supposedly, is an index of the magnitude of the FRT) is nearly independent of length in a hypertonic muscle.

The effect of hypertonicity on metabolic rate. The results of the present paper give no direct indication as to what is happening to the metabolic rate. Although the FRT may be generated by metabolic reactions, its increase with hypertonicity does not necessarily mean that the rate of these reactions also increases, because a decrease of the rate of the relaxation process, rather than an increase in the rate of the process of tension generation, could be the factor responsible for raising the tension level. On the other hand it has been shown that the resting metabolic rate, as measured by heat production (Yamada, 1968), by oxygen consumption (Sekine, Iijima, Genba, Tanaka & Kanai, 1957) and by the break-down of phosphate esters (Daemers-Lambert, Debrun, Dethier & Manil, 1966), is increased substantially in a muscle which is treated with a hypertonic solution. Though this could be due to processes which are quite independent of the FRT-generating mechanism, these results are of great interest in the present discussion, and further correlation between the resting tension and the metabolic rate should be sought.

Why does an increase of tonicity have the observed effects? It has been shown that when a resting muscle is stretched, and the filament surfaces come closer together, the FRT and the tension-holding power of the SREC (when expressed in terms of unit length of overlap) both increase. This is not particularly surprising. More remarkable is the demonstration that the *concentration of non-ionic solutes* in the sarcoplasmic fluid is a very important factor in controlling the magnitude of the effects. It may be suggested that this could be due to some 'physical' action of the soluble protein in potentiating the cross-linking process. Any theory, to explain the contractile process, which involves movement of rigid filaments by the action of flexible cross-bridges, has to make the assumption that the cross-bridges are capable of storing potential energy at some stage of the cycle, by being forcibly stressed against their elastic resistance before making connexion, in the appropriate direction, with the actin filaments. It is

conceivable that this imparting of potential energy to the cross-bridges depends on a direct mechanical coupling by 'viscous' forces produced, perhaps, by local movement of the sarcoplasmic fluid. The efficiency of the coupling might be greatly increased by raising the concentration of the protein molecules in the sarcoplasmic fluid.

The physiological significance of the filamentary resting tension. It has been suggested that the effects under consideration are due to the activity, in a resting muscle, of a few cross-bridges, which are linked by stable, long-lived, bonds to the actin filaments, and give a permanent tension by being appropriately oriented against their elastic resistance. This is an efficient system for holding a tension indefinitely. But what is its purpose? The obvious answer is that it may serve to pull in the filaments of a passively shortened muscle. It has been shown (p. 659) that, in a normal muscle, the FRT is, in fact, probably large enough to overcome the 'frictional resistance' at a speed up to 1 mm.sec^{-1}. But since there is evidence for the existence of passive elastic connexions (the S filaments) which link the actin filaments of one sarcomere with those of the next, and which might serve the purpose in question, the point has to remain undecided.

It may be true that the FRT has, indeed, no useful mechanical function and that its presence is, in a sense, unavoidable. If it is supposed that, in the interests of metabolic quiescence of the resting muscle, not only the reaction which makes the cross-links but also that which breaks them, must be inhibited as far as possible, then the muscle is bound to be in a state of rather delicate balance, and is liable to develop tension, or even to produce an undesirable contracture, if the 'make' reaction is not sufficiently inhibited. It is possible, therefore, that the FRT is nothing more than a sign of a very slight 'failure' of the inhibition of the formation of new links, but one that is unimportant, and acceptable, under normal physiological conditions.

Catch muscle. It seems possible, from what has been said, that the first result of stimulation of a resting muscle is to cause a break-down of the comparatively stable bonds between the cross-bridges and the actin filaments, which are believed to be responsible for the FRT and for the tension produced during a slow change of length. It is therefore interesting to recall that in certain types of smooth muscle stimulation may cause a relaxation of existing tension without, subsequently, producing a positive tension development. It has been known for many years (Winton, 1937) that the anterior byssus retractor muscle of *Mytilus edulis*, when it is in a state of tonic contraction, can be made to relax by the application of an alternating current stimulus. Also, in the resting *Mytilus* muscle it has been shown that the tension caused by stretching can be partially released by repetitive stimulation (Lowy & Millman, 1963).

Reference should also be made to a brief report by Lowy & Sten-Knudsen (1963) of experiments which have shown that an effect which may be essentially similar to the latency relaxation of striated muscle can be elicited from the anterior byssus retractor of *Mytilus*. The relaxation does not occur in response to a single shock, but it appears after two or more shocks which are spaced in time by a few seconds.

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