THE INFLUENCE OF LENGTH ON THE RESPONSES OF UNSTRIATED MUSCLE TO ELECTRICAL AND CHEMICAL STIMULATION, AND STRETCHING.

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Introduction. The tonic and rhythmic activities of smooth muscle have distracted attention from the simple responses to stimulation approximating more closely to those of striated muscle. In the present enquiry some of the factors influencing the contraction of unstriated muscle under the simplest obtainable conditions are considered, with a view (*a*) to bringing under control the conditions which make for more accurate quantitative data, and (*b*) to defining the limits of a possible analogy between the contractile mechanisms of striated and unstriated muscles.

Two difficulties have to be overcome: first, the anatomical fact that most smooth muscles are formed of a large population of small cells arranged in an irregular network, and that contraction may be associated with a sliding movement between the cells (Grützner(1)); second, physiological variations of tonus and liability to spontaneous contraction. The correlation between the properties of a muscle and those of its constituent fibres is indeterminate unless the fibres are parallel and longitudinally arranged. This anatomical arrangement obtains in the dog's retractor penis, which formed the material of most of the experiments described below. If such a muscle be stimulated under isometric conditions, sliding movements between the cells are extremely unlikely. Fortunately for the present purposes, this muscle in isolation is relatively free of tonus(2), and in a considerable proportion of preparations shows little or no tendency to perform spontaneous movements. It is likely, therefore, that the mechanical properties of such a muscle will be relatively simple functions of the chemical and energetic changes associated with its activity.

Method. Adequate electrical stimulation of smooth muscle presents certain difficulties, chief among which are, first, the relatively strong currents required together with the injury which follows unduly intense stimulation, and second, the factors preventing uniform excitation and simultaneous response of a large population of cells. The second circumstance is of greater importance in isometric than in isotonic contraction: for, if a contraction begins at one part of a muscle before another part, the former will shorten and the latter lengthen, while the lever gives little indication of the tension a fibre might develop at approximately constant length. This effect is greater if, as Brücke (3) and Botazzi (4) claim, the conduction of the excitation wave in the retractor is relatively slow (1–7 mm. per sec.) than it is if de Zilwa's record (5) of simultaneous response of two halves of a muscle to stimulation of one-half be accepted. This consideration is reinforced by analogy with other smooth muscle, and by Brücke's (3) observation showing the difficulty of obtaining monophasic action currents in this muscle (cf. Adrian (6)). Such imperfect continuity of a muscle with respect to an excitation wave would again emphasise the importance of stimulating all the cells at the same moment.

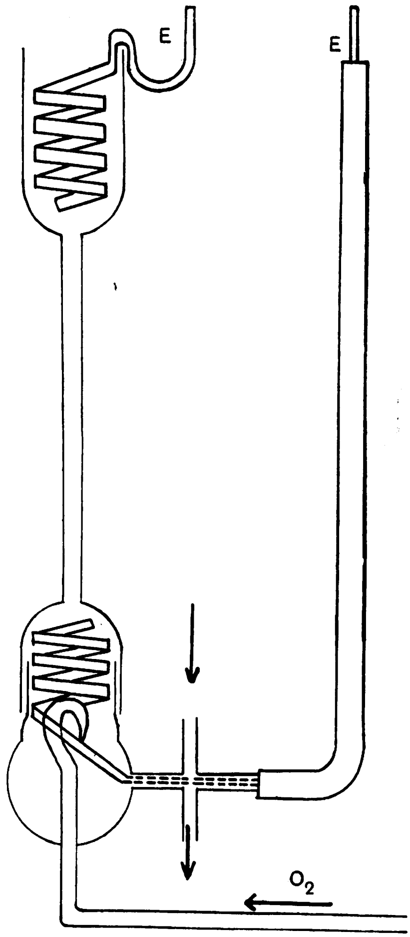

Fig. 1. Stimulation chamber
E, E, wires to electrodes.

Electrical stimulation in a moist chamber yields excellent responses, but tends to induce irreversible change in a retractor, which disturbs the long succession of constant responses to similar stimuli necessary for quantitative experiments. Consequently a stimulation chamber was employed, which consisted of a tube, about three times the cross-section of, and about the same length as a relaxed muscle, opening at each end into a larger tube. The lower end was sealed save for openings allowing ingress of fresh and egress of used solution, a hooked capillary tube for attachment of one end of the muscle and flow of oxygen through its fine orifice, and silver ribbon formed inside into a spiral. The open upper end contained a similar silver spiral. The thread from the upper end of the muscle passed along the axis of the upper electrode to the lever. Thus the current density was increased along the constricted region where the muscle was situated, and as both tube and muscle were of uniform cross-section, the potential gradient along the muscle was also fairly uniform. Moreover, the muscle never came into contact with the electrodes and was not therefore exposed to the electrolytic disturbances apt to occur in their vicinity. The electrodes were heavily coated with silver chloride when direct currents were used, and sometimes also when faradic stimulation was employed. In the latter case some rectification of current occurred when the electrodes were bright, owing, as A. V. Hill (7) pointed out, to their unequal size and dirtiness; the direct current produced appeared to be too small to affect the result. The apparatus was immersed in a large water-bath maintained at a constant temperature.

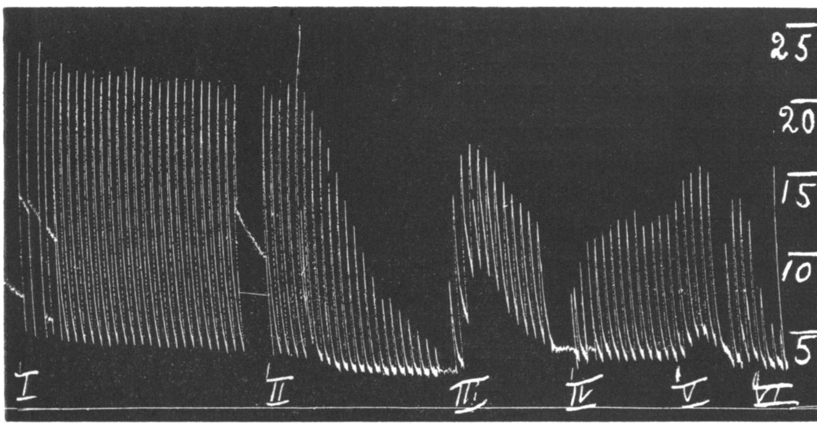

Fig. 2. Isometric responses of a retractor penis (dog) in Burn and Dale's solution at pH 7·6, 35° C. Faradic stimulus, 5 sec. duration, applied once a minute. Scale of tension in grm. wt. on right. *I–II*, about 30 sec. oxygenation between stimuli; *II–III*, no oxygenation; *III–IV*, oxygenation, as in *I–II*; *IV*, 1 hour's rest, with continuous oxygenation; *IV–V*, same as *I–II*; *V–VI*, sodium bicarbonate solution added, oxygenation continued; *VI*, return to normal solution, as in *I–II*.

A continuous stream of fine oxygen bubbles passed up the tube. It was interrupted just before stimulation until about 75 p.c. relaxation. The period of such interruption was not a critical factor influencing responses to stimuli applied every 5 minutes, as was the custom in these experiments. If, however, stimuli were repeated frequently it became an important factor. Fig. 2 shows isometric responses to faradic stimuli applied every minute. They are somewhat irregular owing to slight variation of duration of oxygenation. The effect of complete absence of oxygenation with its concomitant massage of the muscle is shown at *II*, which is soon followed by a steep fatigue curve. The strikingly incomplete recovery when oxygenation is re-started is also shown.

Phosphate-buffered solutions were not found to allow undiminished responses over long periods. A carbonate buffered solution, of the composition described by Burn and Dale(8), was therefore used, and an attempt made to reduce the effective changes of hydrogen-ion concentration by replacing the solution immediately before each stimulation.

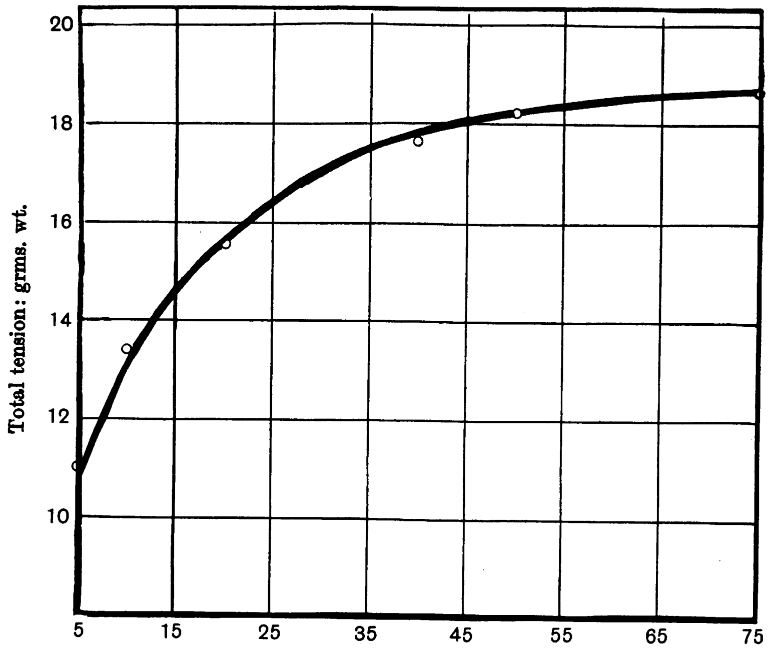

Fig. 3. Tension developed by retractor penis as a function of frequency of interruption of a faradic stimulus of 5 sec. duration. Points on the curve are the means of values obtained at increasing and decreasing frequencies respectively.

The reputation of smooth muscle for capricious response suggested an examination of the relation between tension developed and the strength, frequency, or duration of the stimulus, whether galvanic or faradic: no discontinuity was found. The influence of frequency of faradic stimulation is shown in Fig. 3: the curve allays the suspicion that certain frequencies might produce inhibition, and records a response increasing with frequency of interruption up to 75 per sec. in a tissue with slow general time relations. Fig. 4, showing the influence of temperature, indicates continuity and the region of optimum response. Increasing temperature induces, within limits, increased responses, as is the case with tetanic contraction of striated muscle. Associated variation of initial tension, however, may here contribute to modification of the responses.

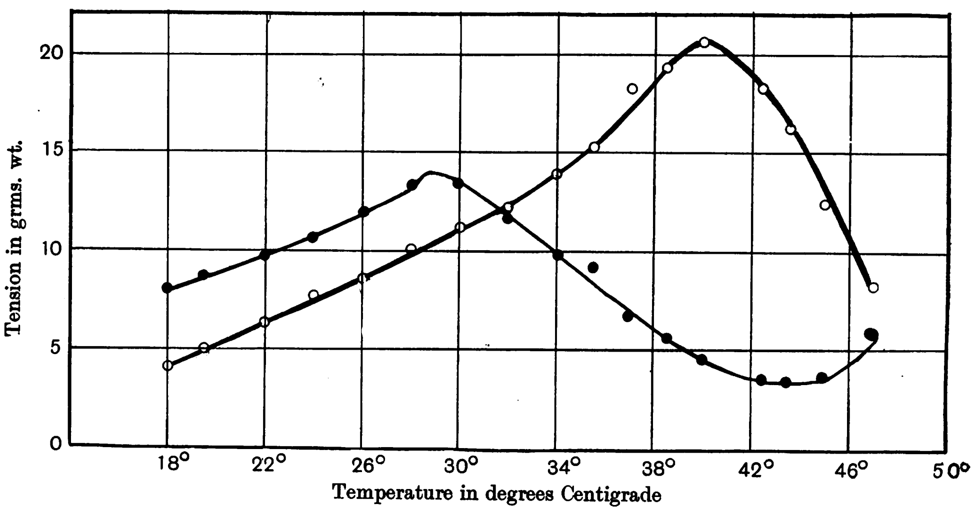

Fig. 4. The influence of warming (i) on the initial tension (dark points), and (ii) on the tension developed on faradic stimulation (circles). (Retractor penis, stimulated for 3 sec. every 5 minutes.)

Tension levers of various strengths were used. One with a long light bamboo pointer, giving about 1 cm. excursion for 10 grm. wt. was found convenient. It allowed shortening of the order of 1 mm. in a full contraction. Observations were made on the reduction of response, when a muscle was stimulated at a particular length, caused by shortening at various uniform velocities. They indicated that truly isometric tensions would exceed those recorded by such a lever by 5–10 p.c.

The influence of initial *length* of muscle on its response is discussed later. The independent influence, however, of initial *tension* also is of importance in smooth muscle. It is difficult to isolate changes of initial tension from concomitant changes of environment; but at a given length, increase of initial tension appears to reduce the response to stimulation in experiments involving the slow fall of tension after a sudden stretch, or the rise of tension due to chemical or electrical stimulation.

Spontaneous contractions, slow variations in tone, and simple responses to stimulation, retain about the same quantitative relations, whether manifested as changes of isometric tension or of isotonic length.

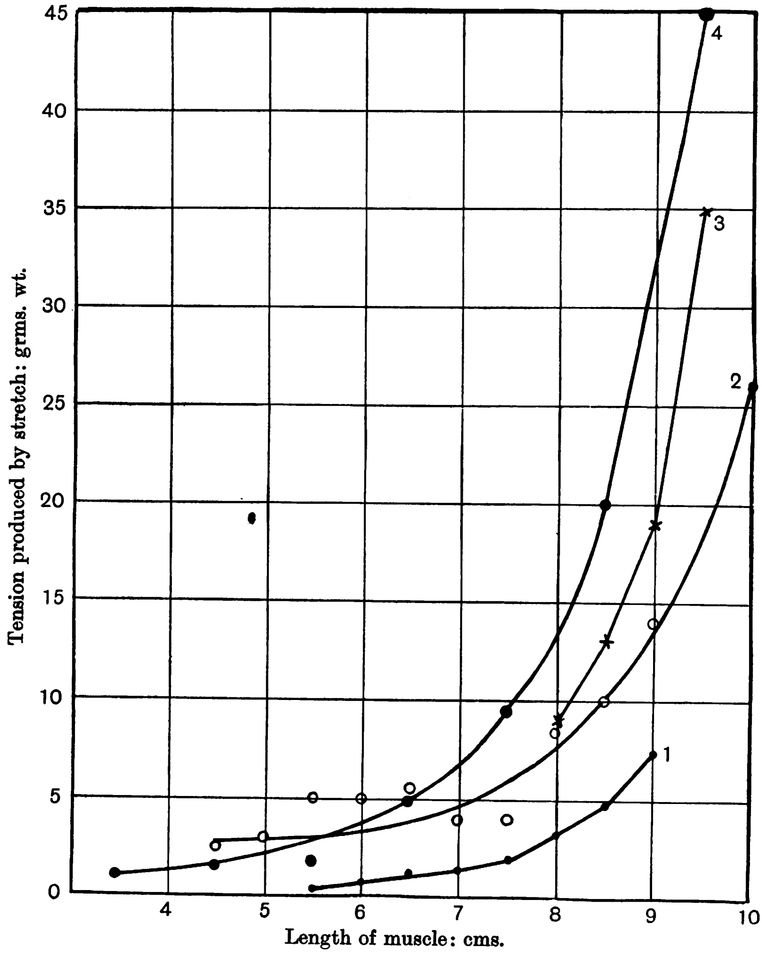

Fig. 5. The tension produced by sudden stretching at varying lengths of four different retractors. Curves 1–4 represent stretches of 1, 2, 5 and 10 mm. respectively. (Muscle 4 was longer than the others, and 3 cm. has been subtracted from each value of its length for inclusion in the figure.)

The influence of length on the reaction to stretching. The curves shown in Fig. 5 give a rough indication of the relation of longitudinal stress to strain at varying lengths of the unstimulated muscle. A crude technique only was employed, which involved rapid lengthening of the muscle by turning a handle manually. The tension produced varied with the speed of stretching, which was kept fairly constant, by adopting a rhythmical procedure regulated by metronome.

The curves derive from experiments on different muscles, and are not reduced to a standard length. They show a general resemblance, within the region shorter than the length of maximal response to stimulation, between the effects on the tension (i) of stretching, and (ii) of stimulation. They demonstrate, further, the increase of longitudinal elasticity with length. This is again shown, on a slower time scale, in the lower tension-length curve shown in Fig. 8, where the slope increases with length. The muscles were in each case stretched during a period of $\frac{1}{5}$–$\frac{1}{2}$ second, maintained at the greater length for 10 secs., and returned to the original length. Successive reactions to the same stretch showed no significant variation, if the initial length, and the rate of stretching were kept constant.

The retractor penis is less readily excited by mechanical disturbance than most plain muscles: it will, for example, in the circumstances obtaining in these experiments, show no contraction superimposed on a sudden stretch or after pinching with forceps.

The influence of length on the response to stimulation. A defect of the apparatus described above is that an electrical stimulus necessarily varies with the length to which the muscle is stretched, owing (1) to a reduction of the proportion occupied by the muscle of the cross-section of the tube, (2) to increase of the length of the potential fall, (3) to reduction of muscle substance directly stimulated, and introduction of phenomena due to slow conduction of excitation, if some of the muscle inadvertently emerges from the narrow to the wider portion. Analogous errors affect stimulation in a moist chamber, or by the application of drugs. It was not found possible to circumvent all these defects. An attempt was made to prevent them from vitiating the results by using a variety of tubes, and by comparing responses to electrical and chemical stimulation. The results as a whole suggest that the inevitable errors are not large enough to mask the essential correlations.

The muscle was attached at its lower end to the stimulation chamber as described above, and the whole of this chamber was fixed by clamps to a stage, the vertical movement of which was controlled by a pulley connected through gearing to an electric motor. The velocity of the vertical movement could be adjusted and measured. The upper end of the muscle was attached to a stationary tension lever recording on smoked paper. In earlier experiments, variation of length of the muscle was achieved by moving the lever 0·5 cm. at a time, as in the work on striated muscle, but such sudden stretching or shortening produced large changes in tension. These were transient, but disturbed the state of equilibrium, interrupted only by stimulation, in which consistent experimental results were best obtained. The rate of change of length

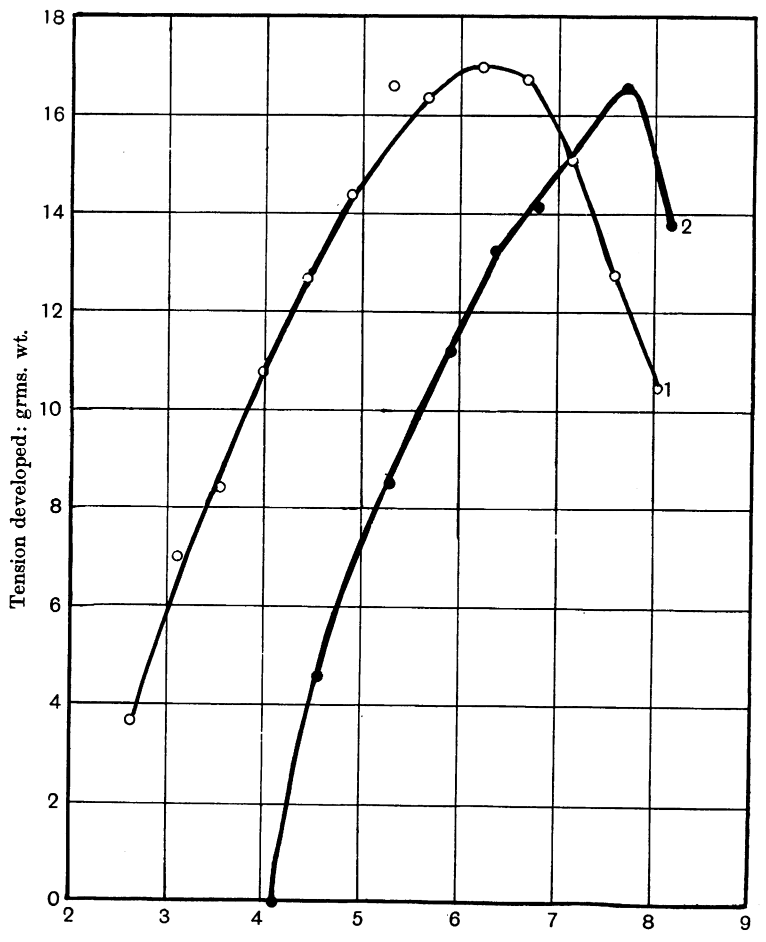

Fig. 6. Tension developed by retractor penis when stimulated at different lengths. Curve 1, muscle stretched at 5·4 cm. per hour (circles); curve 2, muscle allowed to shorten at 5·4 cm. per hour (dark points). Faradic stimuli, 5 sec. Dale's solution, 36° C. 40 hours since death of dog.

varied from about 5 to 8 cm. per hour in different experiments, and the reduction or increase of tension developed on stimulation, due to the continuous change of length, was a measurable experimental error, usually of the order of 1 p.c.

Eight retractors were examined, each at a series of different lengths. Galvanic and faradic stimuli of all durations gave similar results. Fig. 6 shows a typical example of responses to a submaximal faradic stimulus, applied at intervals, while first stretching a muscle to beyond its optimum length, and then releasing. The irreversible increase of functional length of the muscle due to over-stretching is evident. Fig. 7 shows responses both to electrical and to chemical stimulation, during (1) the release following an initial stretch, and (2) a second stretch. The maximum tension for stimulation with adrenalin is at a greater length than that for electrical stimulation and the curves for the former are steeper than those for the latter, differences to which little significance should be attached in view of the errors involved in the method. It will be noticed that the tension developed is not a linear function of the length, though the total tension exerted by the stimulated muscle approaches more closely to this simple relation, as shown in Fig. 8.

The property of unstriated muscle described in this section has an interesting application to the behaviour of the parturient uterus, for it has seemed a curious fact that the muscle of the fundus and cervical canal should be in mechanical equilibrium both before and after expulsion of the fœtus, and that in certain mammals such as rats, the thin distended portions of the uterus should be capable of expelling their contents against the resistance of adjacent stouter portions of the muscle. The profound influence of extension upon the tension which a muscle exerts in response to a sudden stimulus may again be seen in the responses of the guinea-pig's uterus to pituitary extracts, shown in Fig. 9. In order more vividly to illustrate the sufficiency of this mechanism, a horn of a guinea-pig's uterus was attached by its apex to a lower rigid support, while threads were connected to its centre and to its vaginal end, and attached each to a light isotonic lever. The load on the central lever was now increased, so that the ovarian half only of the uterus was stretched for about an hour. The load was then again reduced to its original value, and about half an hour allowed for equilibrium to be established. The lever connected to the vaginal end was then fixed rigidly, so that the length of the entire uterus was kept constant, but any movement of the central segment could be observed. Pituitary extract now induced considerable movement of the central segment, in the sense of shortening of the previously stretched half. This effect was reversible, and elicited by successive doses of the extract.

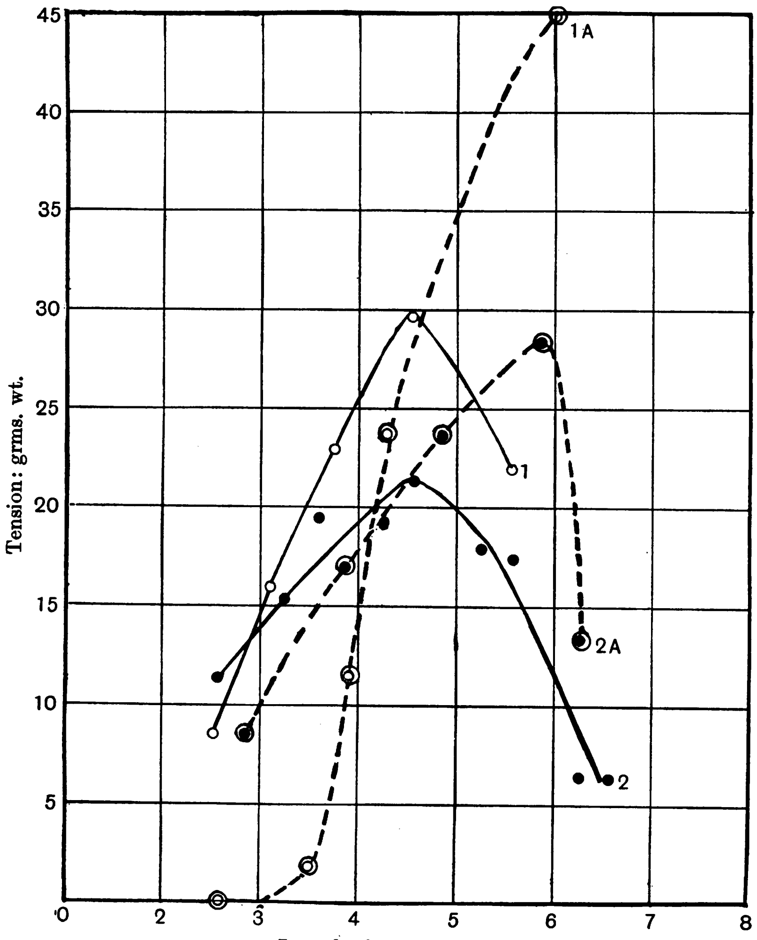

Fig. 7. Tension developed by retractor penis when stimulated at different lengths. Curves 1 and 1*A* shortening at 4 cm. per hour, after a previous stretch (circles); curves 2 and 2*A* subsequent stretching at 4 cm. per hour (black points). Continuous lines 1 and 2 show responses to faradic stimulation, broken lines 1*A* and 2*A* those to 1 in 10^6 adrenalin.

It may be recalled that increasing extension itself tends to produce more frequent auto-excitation (Brücke(3)), and increased response to stimulation with stretching might therefore be due to some form of summation of stimuli. Chemical or other stimulation preceding a superimposed electrical stimulus at a given length tends, however, to reduce the response to the latter; and the influence of excitation due to stretching should thus be in the sense of reduction of responses to stimuli applied at increasing lengths.

The range of length within which muscle responds. Smooth muscle develops effective tension on stimulation throughout a great range of length. Indeed, while skeletal muscles are often described as functionally almost isometric, unstriated organs approach more closely to isotonic

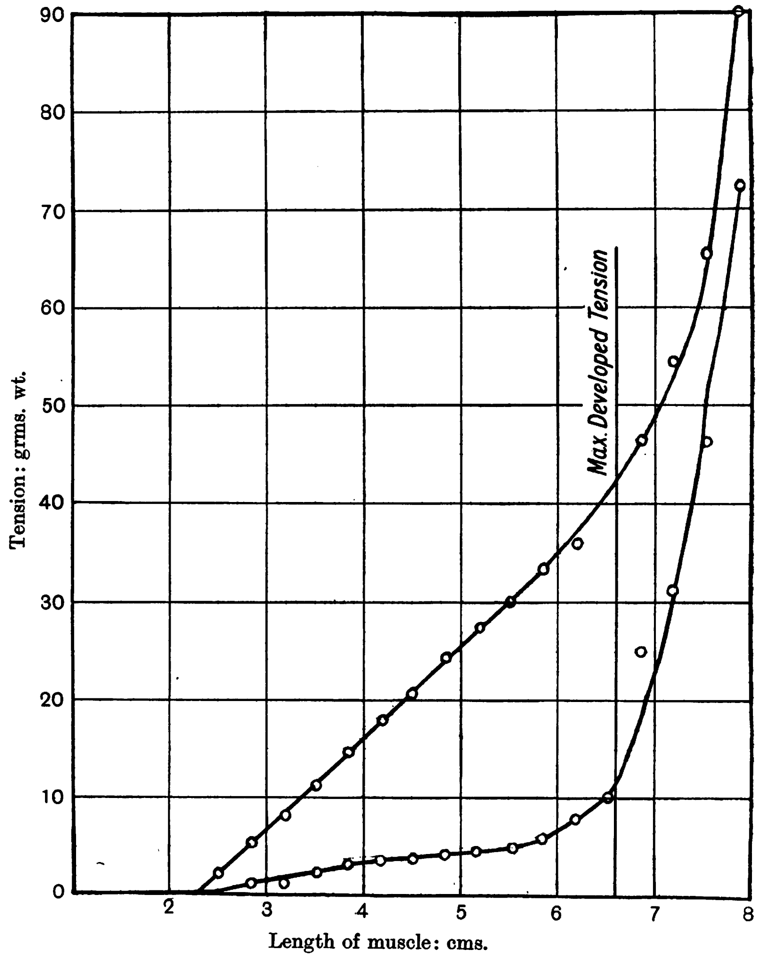

Fig. 8. Tensions at various lengths of a stimulated (upper curve) and unstimulated (lower curve) retractor penis, stretched at 4 cm. per hour. Faradic stimuli, 5 sec. duration, applied every 5 minutes at 36° C. This is the first muscle of Table I.

conditions. It seemed interesting, therefore, to define this characteristic quantitatively.

A muscle stretched beyond its length of maximal response undergoes irreversible changes, as shown in Fig. 6. It is therefore more expedient experimentally, and probably of greater physiological significance, to confine measurements to lengths shorter than this value, and to adopt this length as the standard natural length. If a muscle be stimulated at different lengths, and the tension of stimulated and unstimulated muscle respectively be plotted against the length, as in Figs. 8 and 9, the ratio $W_m/T_m l_m$ (where W_m is the area between the curves up to the length of maximal response, T_m is the maximal tension developed at the optimal length l_m) is dimensionally a number, and may conveniently be taken to represent the relative range of effective response. Fig. 8 shows the variation of tension with length of a retractor penis, (i) unstimulated, and (ii) electrically stimulated. Fig. 9 gives similar particulars for a guinea-pig's uterus stimulated with pituitary extract.

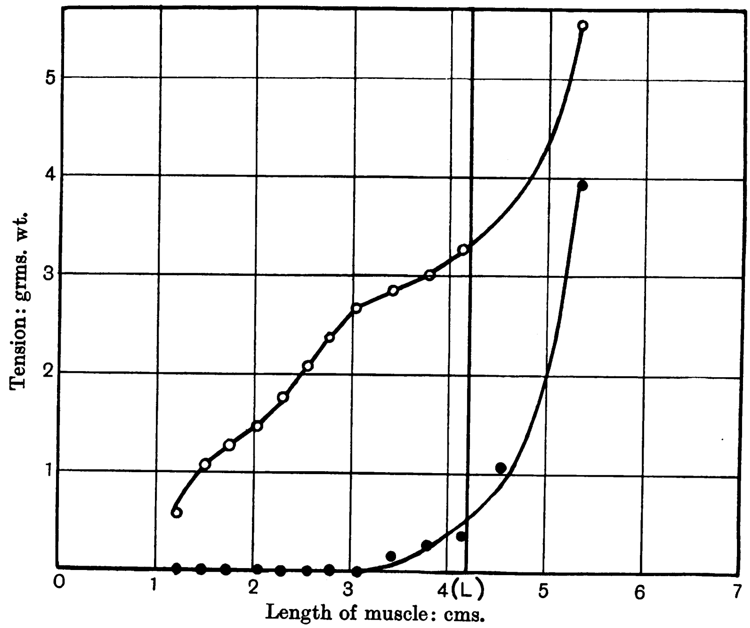

Fig. 9. Tensions at various lengths of a stimulated (upper curve), and unstimulated (lower curve) uterus of guinea-pig, stretched at 2·2 cm. per hour. Stimulus = maximal dose of post-pituitary extract Burn and Dale's solution, 37° C.

The table gives values for a number of muscles treated in much the same way—all but the uterus consisting of parallel fibres and being electrically stimulated. The rectus abdominis of the frog was chosen as representative of striated muscle because it resembles smooth muscle in some of its properties, and notably in its considerable capacity of changing its length without great change of tension in an intact animal. This muscle, therefore, seemed likely to reveal continuity between the two classes of tissues if such existed. Values for its range of effective response are given in Table I, which, though somewhat lower, do indeed approach those given for smooth muscle. The classes of tissue defined by cross-striation and by short range of functional length, respectively, are therefore probably not co-terminous. Considerable reduction of this range of activity takes place in the course of a long experiment; it exceeds the associated diminution of tension development.

TABLE I.

Muscle	Area W_m grm. cm.	Tension T_m grm. wt.	Length l_m cm.	$W_m/T_m l_m$
Retractor penis (dog):				
(1) Stretched 4 cm./hr.	68·3	31·0	6·6	0·33
Retractor penis (dog):				
(1) Stretched 5·4 cm./hr.	46·3	17·0	6·2	0·44
(2) Released 5·4 cm./hr.	36·9	16·6	7·6	0·29
Retractor penis (dog):				
(1) Released 8·4 cm./hr.	65·2	15·8	9·7	0·42
(2) Stretched 8·4 cm./hr.	40·2	13·0	8·3	0·37
Rectus abdominis (frog):				
(1) Stretched 10·0 cm./hr.	10·0	9·15	3·7	0·30
(2) Released 10·0 cm./hr.	11·5	14·3	3·9	0·21
Uterus (guinea-pig):				
(1) Stretched	5·5	2·9	4·2	0·45

l_m is that length at which maximal development of tension occurs; T_m is the maximal developed tension; W_m is the area of the tension-length curve up to length l_m.

The relation between the area (W_m) mentioned above, and the theoretical maximal work deserves brief attention. Experiments on the theoretical maximal work in striated muscles differ from those here described, in that stimulation in the former is usually carried out at the same initial length. In the tetanic contractions employed by Hill (9), the muscle shortens and develops its maximal tension at the new length. The relation between length and response may be affected, as in the above experiments, either by the contractile or excitatory mechanisms, or by the actual change of effective stimulus associated with change of electrical resistance of the shortened muscle. The use of supra-maximal stimuli in striated muscle obviates errors due to the latter consideration, whereas the inevitable use of submaximal stimuli with easily damaged smooth muscle emphasises its importance. The instantaneous stimuli employed by Meyerhof(10), on the other hand, ensure constancy of excitation, but tensions developed at different lengths then represent different, and not necessarily maximal, points along the isometric tension-time curve. Losses of energy due to internal friction thus reduce the tension measured. This conception of the theoretical maximal work which a muscle can perform in response to a particular stimulus does not, however, imply supra-maximal stimulation, and experiments are in progress intended to determine the quantity for smooth muscle in this way. In the meantime it may be mentioned that the areas measured above for the retractor penis, if taken as of the same order as the heat production of the muscles, would correspond to a rise of temperature of the order of 0·003° C. Such an area, however, represented about six times the actual mechanical external work done in shortening at a favourable uniform velocity in a particular muscle which developed the same isometric tension.

Summary.

1. A long succession of constant responses under approximately isometric conditions can be obtained with the retractor penis in the stimulation chamber described.

2. The influence of a number of factors on the tension developed was examined, and the best conditions for uniform responses determined.

3. The longitudinal extensibility of unstimulated smooth muscle decreases with length.

4. The isometric tension developed in response to electrical or chemical stimulation increases with length up to a maximal value, and then decreases. The total tension of a stimulated retractor approaches a linear function of the length more closely than does the tension developed.

5. The mechanism of expulsion of a fœtus by the uterus is discussed.

6. The range of length within which muscles respond effectively to stimulation is considered, and found to be of the same order in certain striated and unstriated muscles.

7. The theoretical maximal work of smooth muscle is considered in relation to measurements of tension development at different lengths.

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REFERENCES.

1. Grützner. Ergeb. der Physiol. 3. p. 13. 1904.
2. Evans and Underhill. This Journ. 58. p. 1. 1923.
3. Brücke. Pflüger's Arch. 133. p. 313. 1910.
4. Botazzi. Arch. Ital. de Biol. 65. p. 17. 1916.
5. de Zilwa. This Journ. 27. p. 200. 1901.
6. Adrian. Ibid. 60. p. 303. 1925.
7. Hill. Ibid. 46. p. xvii. 1913.
8. Burn and Dale. M.R.C. Report, No. 69. 1922.
9. Hill. This Journ. 60. p. 237. 1925.
10. Meyerhof. Pflüger's Arch. 191. p. 128. 1921.