MECHANICAL STUDIES ON THE RETRACTOR BULBI MUSCLE AND ITS MOTOR UNITS IN THE CAT

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SUMMARY

1. For a description of some of the mechanical properties of the retractor bulbi muscle in the cat, the isometric tension development was studied in one of the four slips of the muscle and in single retractor bulbi motor units.

2. The motor units and the muscle were activated by stimulating their motoneurones of origin in the abducens nucleus.

3. All twenty-eight motor units studied were of the same fast, twitch type, which fatigued quickly. Similarities between the motor units and the muscle with regard to isometric tension development strongly suggest that the retractor bulbi is composed exclusively of this type of motor unit.

4. On an average the motor unit had a twitch rise time of 9·2 msec and a half-decay time of 11·4 msec. The twitch tension was 36·7 mg. At stimulation with 175 pulses/sec or above the tetanus fused. The maximal tetanic tension developed was 440 mg and the maximal rate of tension rise was 24·5 g/sec. To prolonged tetanic stimulation most of the units fatigued completely within 10–15 sec.

5. In a slip of the muscle similar values were obtained for contraction time, half-decay time, fusion frequency and fatigability. Tetanic tension reached 11·7 g and the maximal speed of tetanic contraction was 255 g/sec.

6. As in other extrinsic eye muscles a linear relation was found between length and tension in the activated retractor bulbi. An increase in activation induced a parallel shift in the curves to higher tension values but the slopes of the curves remained unchanged. The average slope value was 1·7 g/mm.

INTRODUCTION

From electrophysiological studies (Bach-y-Rita & Ito, 1965) and morphological studies (Alvarado, Steinacker & Bach-y-Rita, 1967) of the cat's retractor bulbi muscle, it has been suggested that the fibre population of this muscle is rather uniform and made up exclusively of fast twitch fibres. No evidence has been obtained for the existence of slow fibres of the kind seen in the six oculorotary muscles (Bach-y-Rita, Levy & Steinacker, 1967). The retractor bulbi might serve as a prototype of a pure, fast fibre muscle to be used, for instance, in dynamic studies of muscle mechanics (cf. Bach-y-Rita, 1971).

However, for this notion to be valid motor units of the retractor bulbi should be of a single type. One aim of the present study was to test this possibility. A second purpose of the study was to supply data on mechanical properties of the retractor bulbi to be used in dynamic studies.

METHODS

General. Adult cats (2·0–4·5 kg) were anaesthetized with pentobarbitone. The head of the cat was fixed in a Palmer head holder rigidly mounted on an iron base, to which myograph and electrode holders were attached. The back of the animal was lifted; this reduced interference from respiratory movements in the tension recordings. Pneumothorax and artificial respiration were sometimes instituted for the same reason, but also in order to reduce movements of the brain stem during stimulation (see below).

Muscle preparation. Surgical techniques similar to those described by Bach-y-Rita & Ito (1965) and Steinacker & Bach-y-Rita (1968) were used. The muscle was submerged in warm mineral oil, the temperature of which was controlled at 37–38° C in a thermistor circuit with a flashlight bulb as the heating element.

Stimulation. The whole retractor bulbi or motor units thereof were activated in the abducens nucleus which is probably the only site of origin for motoneurons of this muscle (Bach-y-Rita, 1971). This nucleus also supplies the lateral rectus muscle, which was denervated close to the muscle. Stimulation of mid-brain structures reported by Matyushkin (1972) to generate slow motor unit activity in the oculorotary muscles of the rabbit gave no responses in the retractor bulbi of the cat. For the gross stimulation of the whole motor nucleus, a concentric bipolar electrode was used; the outer part was a varnished 26-gauge hypodermic needle and the inner part an insulated copper wire. In control experiments the abducens nerve was stimulated close to the brainstem (for technique see Bach-y-Rita & Murata, 1964). Both types of stimulations gave the same results; the brain stem stimulation evidently activated the retractor bulbi muscle maximally (see also Steinacker & Bach-y-Rita (1968).

Single motor units were activated between a varnish-coated tungsten microelectrode with a tip diameter of 3–10 μm and a silver electrode attached to the neck muscles.

The electrodes were connected to a Grass S8 stimulator through an isolating unit (Grass SIU 478A). Square pulses of about 100 μsec duration were applied. Stimulus strengths were mostly between 0·2 and 1·0 V and never exceeded 5 V.

Tension recording. In the experiments on single motor units, the force sensitive element of the myograph for isometric tension recording was a bare Pixie transducer (no. 8101, Endevco Laboratories). One end of the transducer was clamped to a slider; to the other end a slip of the retractor bulbi was attached with 5-0 surgical silk. The other slips were stretched and tied taut to the head holder so as not to interfere mechanically with the recordings from the activated portion of the retractor bulbi (Steinacker & Bach-y-Rita, 1968). A vernier scale on the slider allowed positioning of the myograph within one tenth of a millimetre. The compliance of this myograph was about 2 μ/g and its natural (undamped) frequency was 2 kHz. The myograph had a linear output in the tension range of 1–2 mg to 10 g.

In the experiments on a whole retractor bulbi slip, a less sensitive myograph was used. It consisted of four strain gauges (SR-4, BLH Electronics, Inc.) glued to a stiff steel spring. The compliance of this myograph was also about 2 μm/g and its undamped resonance frequency was 1 kHz. It was linear from 0·1 to 100 g.

The transducers of both myographs operated as external bridge arms of a Tektronix 3A66 carrier amplifier. The tension signal was fed through a low-pass filter (break frequency 1·0 kHz) and was further amplified in a Tektronix 5A18N DC amplifier and displayed on one beam of a Tektronix 5103N storage oscilloscope.

Electrical recording. Electrical activity from the muscle was recorded bipolarly between 0·5 mm chlorided silver wire electrodes, one resting on the muscle belly, the other on the tendon. Recorded activity was amplified in a differential DC-preamplifier and a Tektronix 5A21N amplifier and displayed on the oscilloscope together with tension and stimulus signals.

RESULTS

The first part of this study deals with the isometric tension development in single motor units (twenty-eight in total) or in one of the four slips of the retractor bulbi. All data were collected with the muscle extended to a position where twitch tension peaked, i.e. the optimal length (see Fig. 7). The data for the whole muscle corresponded closely to those reported by Steinacker & Bach-y-Rita (1968) but are included in this presentation for comparison with similar data from single motor units.

The second part is a description of length–tension relationships in the passive and active muscle. Only steady-state data from the whole muscle will be presented in this paper.

I. Isometric tension development

Twitch responses in a retractor bulbi slip. From the twitch responses to graded stimulation of the motor nerve it is sometimes possible to determine if a muscle contains motor units of different speed of contraction (e.g. Teig, 1972a). In the case of a motor unit population with almost identical mechanical properties, very little change with stimulus strength should occur in the development and decline of the twitch response.

As shown in Fig. 1A, the contraction of the retractor bulbi twitch (measured from start of tension rise to peak tension) and the time for half-

decay of tension (measured from peak tension) stayed constant in a fifteen fold increase in stimulus strength.

Isolation of single motor units. As mentioned in Methods the retractor bulbi motoneurones were stimulated extracellularly with tungsten micro-electrodes. According to Teig (1972*b*) it is feasible in this way to activate single motoneurons in the brainstem, innervating the stapedius and the tensor tympani muscles. The method was found successful also for retractor bulbi motoneurones although careful adjustment of electrode
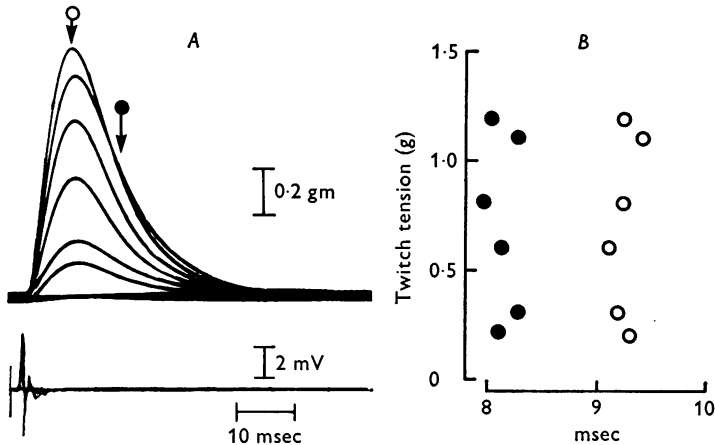

Fig. 1. *A*, superimposed isometric tension responses (upper traces) and electrical responses (lower traces) elicited in a slip of the retractor bulbi by single stimulus pulses of increasing amplitudes from 0·7 to 10 V. The arrows indicate points on the top tension curve for measurements of contraction time (open circle) and half-decay time (filled circle). The contraction time was measured from the start of tension increase to the peak of the twitch, the half-decay time from twitch peak to the point at which half peak tension was reached. Records retouched. *B*, a graph in which contraction time (open circles) and half-decay time (filled circles) has been plotted against response amplitude in the set of twitch tension curves in *A*.

position and stimulus strength was necessary. Electrical recordings were found more sensitive than tension registrations to establish that single motor units had been activated. It has earlier proved possible to detect electrical responses from single twitch units of varying sizes in the super-ficial lumbrical muscles (Appleberg & Emonet-Dénand, 1967) and from twitch and slow units in the inferior oblique muscle (Lennerstrand, 1972). If tension alone had been used to monitor the all-or-nothing response of the retractor bulbi units to the electrical stimulation, in many cases multiple motor unit preparations would wrongly have been regarded as single unit ones.

A refined but much more difficult technique of activating single motor units is that of intracellular stimulation of motoneurones. Such experiments are in progress for units in the lateral rectus muscle. In a few cases also retractor bulbi motoneurones have been activated. The twitch and tetanic characteristics of these motor units were found to fall within the ranges given in Table 1 (S. Goldberg & G. Lennerstrand, unpublished observation).
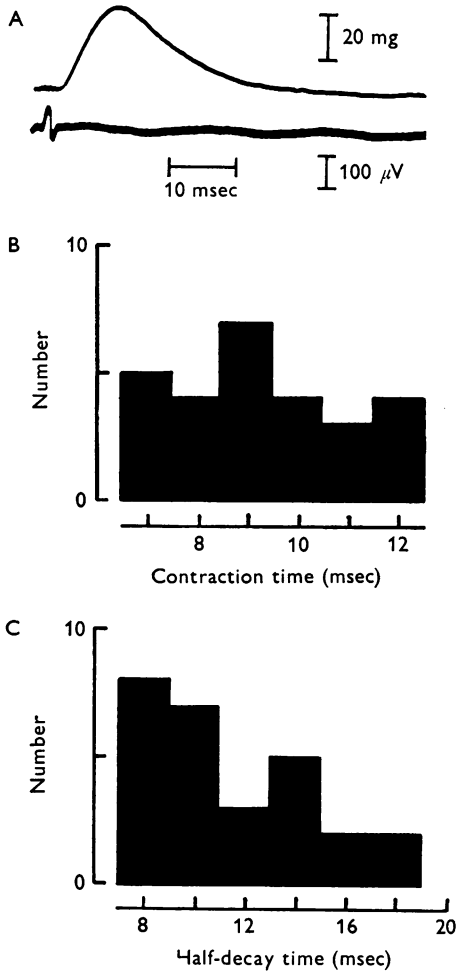

Fig. 2. *A*, the isometric twitch (top) and the corresponding electrical activity (bottom) in an isolated motor unit. The contraction time of this unit was 8 msec and the half-decay time 10 msec. The biphasic electrical response is typical of a single retractor bulbi motor unit. *B* and *C*, histograms showing the distribution of the contraction times (*B*) and the half-decay times (*C*) in a sample of 28 retractor bulbi motor units.

Twitch responses in single motor units. A typical twitch response of a single motor unit is shown in Fig. 2 *A*. In the histograms of Fig. 2 *B* and 2 *C*, the values for contraction time and half-decay time of twenty-eight single motor units have been displayed, and they may be compared with those obtained from a whole slip of muscle in Table 1.

The average twitch tension of single motor units was found to be 36·7 mg (see Table 1).

TABLE 1. Mechanical characteristics of the retractor bulbi (RB) muscle slips and motor units. The data of Steinacker & Bach-y-Rita (1968) in the third column are included for comparison. *t* tests were made comparing motor unit and retractor bulbi slip data for twitch contraction time, twitch half-decay time and fusion frequencies. All showed values of $P < 0.001$, strongly indicating that the two samples of data are from the same population

	Motor units ($n = 28$) Mean $\pm$ S.D. Range	RB slip ($n = 10$) Mean $\pm$ S.D. Range	RB slip Mean; range (Steinacker & Bach-y-Rita)
Twitch contraction time (msec)	9·6 $\pm$ 2·0 7·0–12·5	10·4 $\pm$ 1·6 9·0–14·0	12·7 9·0–18·0
Twitch half-decay time (msec)	11·7 $\pm$ 3·1 8·0–18·0	11·4 $\pm$ 2·0 9·0–16·0	12·9 10·0–16·0
Fusion frequency (pulses/sec)	175 $\pm$ 15 140–200	177 $\pm$ 12·5 160–200	176 120–220
Twitch tension	36·6 $\pm$ 19·0 mg 15–85 mg	0·82 $\pm$ 0·37 g 0·5–1·2 g	0·66 g 0·4–1·3 g
Maximal tetanic tension	442 $\pm$ 170 mg 220–775 mg	12·3 $\pm$ 2·6 g 9·0–15·6 g	10·5 g 7·3–18·6 g

Stimulation with double shocks. In experiments where double shocks of equal duration and magnitude were applied to the preparations (Fig. 3 *A*), the variations in size of the mechanical response to the second pulse was related to the interval between pulses. In Fig. 3 *B* the amplitude of the second response, expressed in percent of the amplitude of the first, is plotted against pulse interval for both the single unit and the retractor bulbi slip in Fig. 3 *A*. The two curves correspond closely.

Fusion frequency. Fig. 4 presents unfused and fused tension responses to tetanic stimulation of a retractor bulbi motor unit. The lowest frequency of stimulation at which the tetanus fused, i.e. fusion frequency, was 175/sec.

The mean value observed for single motor units was 175/sec and for retractor bulbi slips 177/sec (see Table 1).

Maximal tetanic tension. Fig. 5 *A* shows a family of tetanic tension curves from a single motor unit. In Fig. 5 *B* the similar responses from a
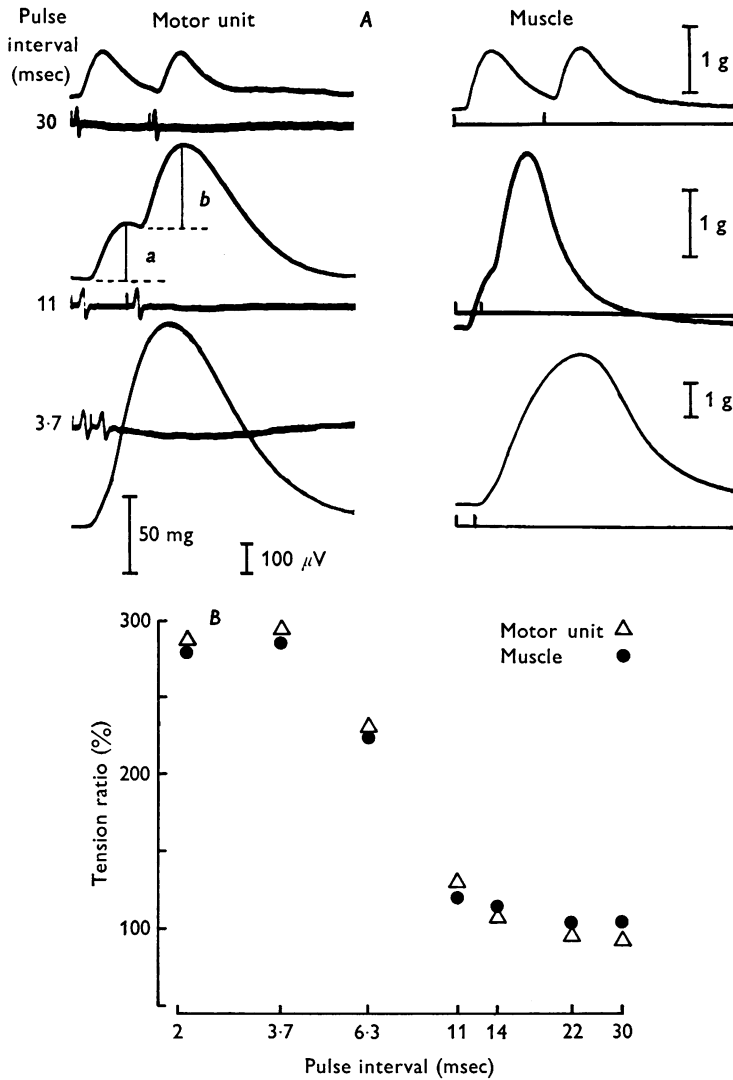
Fig. 3. *A*, activation of a motor unit and a slip of the retractor bulbi with two stimulus pulses of equal amplitude and duration. The interval between the pulses is marked to the left of the traces and is the same in both preparations. Note that the time scale varies from panel to panel. In the motor unit electrical activity was also monitored (lower trace in each section). Electrical records retouched. *B*, graph to show the facilitation of the response to the second stimulus. Ordinate values are peak amplitude ratios of second over first response (*b/a* as marked in *A*) expressed in percent. They are plotted against time interval between stimulus pulses.

slip of the retractor bulbi are displayed. The values of maximal tension to each stimulation in Fig. 5*A* and 5*B* have been plotted against rate of stimulation in Fig. 5*C*. Linearly rising parts of the curves were observed in both cases for inputs between 50 and 150 pulses/sec.

Table 1 shows that the average value for maximal tetanic tension in single units (i.e. peak value of curves such as those in Fig. 5*C*) was 440 mg (range 200–775 mg). For slips of retractor bulbi the corresponding value was 11·7 g (range 9·0–15·6 g).
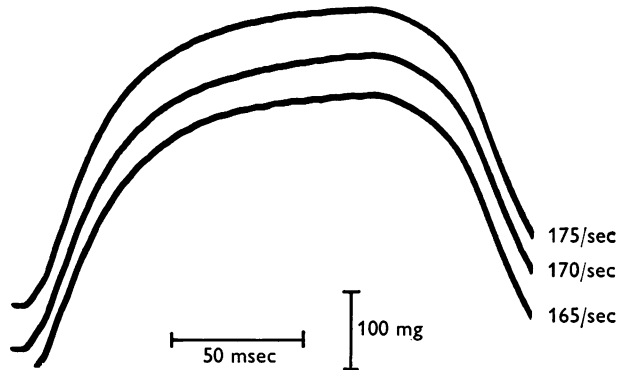

Fig. 4. Recording of tension in a single motor unit during tetanic stimulation at 165, 170 and 175 pulses/sec. A fused tetanus resulted from the 175 pulses/sec stimulation. At the lower stimulations the tension is unfused.

Rate of tension rise. The slope of a tangent fitted to the steepest part of the tension curve was used as a measure on the maximal rate of rise of tension. In Fig. 5*D* the slope values for the unit in Fig. 5*A* and the muscle slip in Fig. 5*B* have been plotted against stimulus frequency. While the curves for maximal tension levelled off before the stimulus frequencies reached 200/sec (Fig. 5*C*), the rate of tension rise continued to increase with inputs up to at least 300/sec (Fig. 5*D*) and sometimes even up to 400–500/sec. Mean values of the rate of tension rise were 24·5 ($\pm$8·1 S.D.) g/sec in the motor units and 255 ($\pm$91 S.D.) g/sec in the retractor bulbi slips.

Fatigability. Stimulations of 30 sec duration at rates between 100 and 200 pulses/sec have been used to test the ability of the retractor bulbi and its motor units to keep up tension during prolonged activations (Fig. 6). At 200 pulses/sec all units and retractor bulbi slips fatigued completely within the period of constant stimulation (Fig. 6*A* and *B*). The residual tension after 30 sec of 100 pulses/sec stimulation never exceeded 5 % of the initial value.

II. Static length–tension relations

Since information on this point seems to be missing for the retractor bulbi muscle, static data on length–tension relationships were acquired. A slip of the muscle was moved to different lengths, starting at an arbitrary zero length. At each position it was stimulated with single shocks or tetanic bursts of supramaximal stimulus strength.

The curve in Fig. 7 labelled 'twitch' represents twitch tension amplitudes at different extensions of the muscle. The extension at peak twitch tension has been used as the working point in the isometric studies (see above).
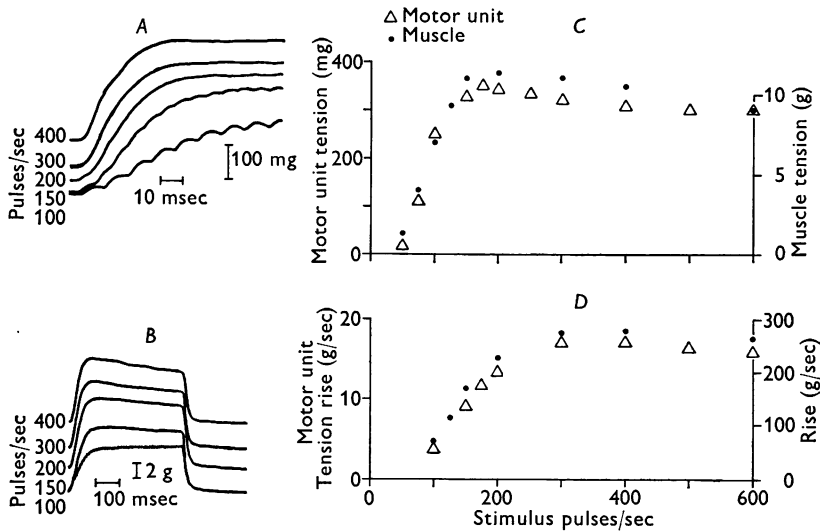
Fig. 5. *A*, the development of isometric tetanic tension in a motor unit. Tetanic stimulation at the frequencies marked was initiated at the beginning of each trace and continued throughout the recording. The maximal rate of tension rise was calculated from the slope of a tangent to the steepest part of the curve. The base line has been shifted upwards in the three upper curves. The curves are tracings of original records. *B*, tetanic tension curves from a slip of the retractor bulbi. Rates of stimulation are marked at the traces. The base line has been shifted upwards for the three upper traces. *C*, a plot of maximal tetanic tension against stimulus frequency for the single unit in *A* and the retractor bulbi slip in *B*. Note that tension values for the lowest rates of activation had to be measured in longer stimulations than shown in *A* and *B* in order for the tension to fully reach a steady state. In both the single unit and in the slip, tetanic tension was maximal at the fusion frequency of stimulation, i.e. 175/sec for the motor unit and 190/sec for the retractor bulbi slip. *D*, a plot of maximal rate of tension rise against stimulus frequency for the same motor unit and retractor bulbi slip. Peaking in this case occurred at a higher stimulus rate (400/sec) than for the tetanic tension in *C*.

The curve relating length and tension of the passive muscle was approximately exponential (Fig. 7). With increasing level of graded activation of the muscle, the length–tension relation became approximately linear. Variations in level of activation induced a parallel shift in the curves as in other extrinsic eye muscles (Collins, 1971). The 'gain factor', i.e. the slope of the curves averaged 1·7 (range 1·6–2·0) g/mm for 200 pulses/sec of stimulation in eight muscles and was very much the same at 100 pulses/sec of stimulation.
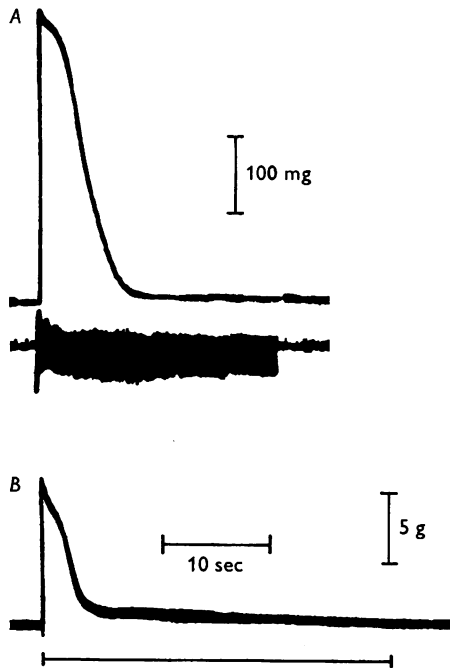

Fig. 6. Complete fatigue in a motor unit (*A*) and in a retractor bulbi slip (*B*) exposed to continuous stimulation at 200 pulses/sec. Duration of stimulation indicated by e.m.g. in *A* (lower trace) and by stimulus bar in *B*.

DISCUSSION

It has been demonstrated that probably all motor units of the retractor bulbi are of the same fast, twitch type. Part of the support for this statement came from the invariability with stimulus strength observed in the twitch time characteristics of the whole retractor bulbi muscle. The variations shown in Fig. 1 *B* seems too small and random to be explained by recruitment of separate groups of motor units at different stimulus levels. They are more likely to be due to variations in the muscle excitation processes and to errors of measurement. Therefore, the results would

indicate that the same type of motor unit was recruited in the retractor bulbi as the stimulus increased. However, a small population of faster or slower units might remain undetected, as was found when the intercostal (Andersen & Sears, 1964) or the anterior tibial muscles were activated (Gordon & Phillips, 1953), and this necessitated other types of tests.
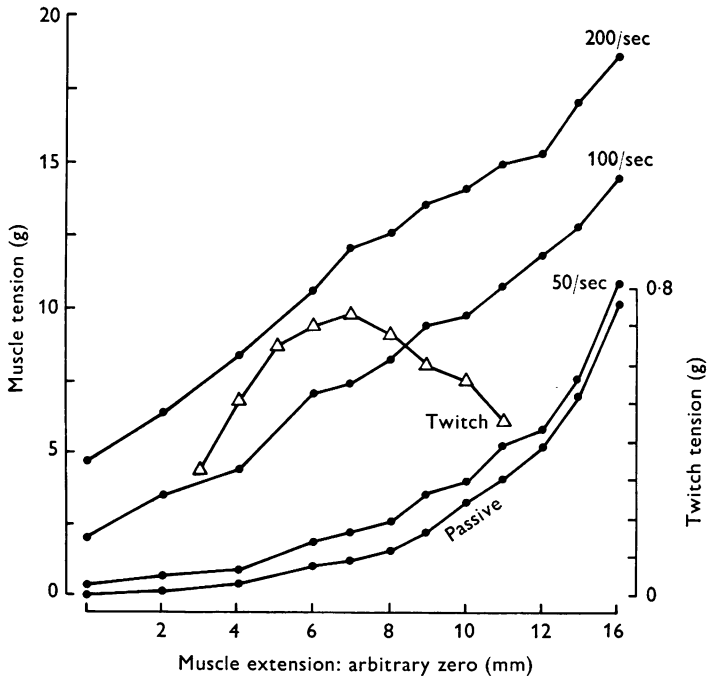

Fig. 7. Static length–tension curves recorded in a slip of the retractor bulbi muscle. In the same graph are plotted the variations with muscle extension in twitch tension amplitude and in total tetanic tension to graded stimulation. Since the resting length of the muscle was not deter-mined, zero muscle extension is arbitrary. The working point in the isomet-ric studies has been the muscle extension at which twitch tension was maximal. In the passive state the length–tension curves were exponential in shape. To tetanic stimulation the curves became linear and parallel.

These consisted of comparisons between individual motor units and the retractor bulbi slip in twitch time characteristics (Fig. 2 and Table 1), behaviour to double shock stimulation (Fig. 3), fusion frequency (Table 1), and variation in tetanic tension (Fig. 5*C*) and in rate of tension rise (Fig. 5*D*) with stimulus frequency.

These tests revealed a very close resemblance in behaviour between the muscle and its motor units, strongly indicating that the retractor bulbi muscle is composed of only one type of motor unit. This idea was further

supported by the results of statistical analysis of the measurements collected in Table 1. It should be noted in the Table that our results on the retractor bulbi slips fell within the same range as those of Steinacker & Bach-y-Rita (1968). By means of *t* tests it was shown that the unit and retractor bulbi slip samples of twitch contraction times, twitch half-decay time and fusion frequency most probably derived from the same statistical population (*P* values given in Table 1).

It is known from the work of Henneman and collaborators (Henneman & Olson, 1965; Wuerker, McPhedran & Henneman, 1965) and Appelberg & Emonet-Dénand (1967) that fast twitch contraction in motor units is positively correlated to high fusion frequency, twitch tension and tetanic tension. For the retractor bulbi units no similar correlation was found in the mechanical parameters, when tested for rank order correlation. Thus, the variations seen in the quantities of Table 1 do not conceal any consistent variations among the units compatible with a further grouping of them, but these variations would represent biological deviations within a single group of fast motor units.

The fast motor units in the retractor bulbi showed a speed of contraction in twitch and tetanus that put them as intermediates to the fast and the slow units of the globe rotating muscles (Steinacker & Bach-y-Rita, 1968; G. Lennerstrand, in preparation). The retractor bulbi units did not possess the high resistance to fatigue found in most fast units of the inferior oblique muscle (G. Lennerstrand, in preparation). In this respect they resemble fast units in leg muscles (Wuerker *et al.* 1965; Edström & Kugelberg, 1968), and probably closest to the fast contracting and fast fatiguing unit described by Burke, Levine, Zajac III, Tsairis & Engel (1971). There are also morphological similarities between muscle fibres of the retractor bulbi and the fibres of the fast motor units in the leg muscles (Alvarado *et al.* 1967). The proposed function of the retractor bulbi muscle is to protect the eye by pulling it into the orbit and pushing up the nictitating membrane (Motais, 1885; Bach-y-Rita, 1971). There might be small need for highly fatigue-resistant units in the retractor bulbi since this muscle probably has rather restricted periods of activity.

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