# FORCE:VELOCITY PROPERTIES OF KITTEN MUSCLES

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## SUMMARY

1. The characteristics of isometric contractions and force:velocity properties of the extensor digitorum longus (EDL) and soleus (SOL) muscles of neonatal kittens were determined *in situ*.

2. The mean contraction time is 51 msec for EDL and 70 msec for SOL and the half-relaxation time is 51 msec for EDL and 109 msec for SOL.

3. The average maximum isometric tetanic tension per unit cross-sectional area of muscle is 1·27 kg/cm² for EDL and 1·17 kg/cm² for SOL.

4. The average twitch:tetanus ratio is 0·28 for EDL and 0·119 for SOL; the low value for SOL was found for both indirect and direct stimulation.

5. The average maximum speed of shortening of a sarcomere is 22·8 $\mu$/sec for EDL and 12·7 $\mu$/sec for SOL.

6. These properties of neonatal muscles are compared with those of adult cat muscles and discussed in connexion with differentiation of mammalian muscles into fast and slow types.

## INTRODUCTION

In some mammals all the limb muscles are slow at birth. Subsequent changes, which occur during the first few weeks of life, lead to differentiation of fast muscles and slow muscles. The pattern of differentiation, as indicated by changes in the time course of isometric contractions, is similar in cat, rabbit, rat and mouse (Banu, 1922; Denny-Brown, 1929; Koschtojanz & Rjabinowskaja, 1935; Buller, Eccles & Eccles, 1960; Close, 1964, 1965*b*; Buller & Lewis, 1965). A more detailed description has been obtained for rat and mouse muscles by determining the relation between speed of shortening and load in isotonic contractions for a fast muscle, the extensor digitorum longus (EDL), and a slow muscle, the soleus (SOL), at different stages of development (Close, 1964, 1965*a*). In these rodents the force:velocity properties of EDL and SOL are virtually identical at birth, thereafter the speed of shortening of sarcomeres of EDL increases two to threefold within 3–4 weeks, whereas SOL undergoes

little or no change in this respect. It has been suggested that this increase in intrinsic speed of shortening is the principal change leading to differentiation of the two kinds of muscles in rat and mouse (Close, 1965*b*). In view of the similarity between rodents and some other mammals in the pattern of changes in the isometric twitch contraction time during development, the possibility arose that differentiation of fast and slow muscles is brought about in the same way in different animals through changes in the speed of shortening of sarcomeres of the fast muscles. This possibility has been investigated for the cat in the present work. The force:velocity properties of a fast muscle and a slow muscle have been determined for new-born kittens and compared with those reported previously for adult cats.

## METHODS

The dynamic properties of the EDL and SOL muscles of one limb were examined in three 1- to 2-day-old kittens. The animals were anaesthetized with 20 mg pentobarbital sodium/kg body wt. injected intraperitoneally, followed by one quarter of the initial dose every 1–2 hr. The preparations were set up with the muscles *in situ* and with the major blood vessels supplying the muscles intact. The lower limb was immersed in about 150 ml. of Ringer fluid (NaCl 137 mM; KCl 5 mM; $CaCl_2$ 2 mM; $MgCl_2$ 1 mM; $NaH_2PO_4$ 1 mM; $NaHCO_3$ 2 g/l.; glucose 2 g/l.) which was bubbled continuously with 95 % $O_2$ and 5 % $CO_2$ and maintained at temperatures between 34·5° and 36·0° C. The muscles were stimulated indirectly with 20 $\mu$sec pulses through platinum wire electrodes on the tibial nerve for SOL and the common peroneal nerve for EDL. Reflex activation of the muscles was prevented by cutting the sciatic nerve central to the point of stimulation, and mechanical interference through contraction of neighbouring muscles was avoided by transecting the nerves to all muscles of the lower limb except SOL and EDL. The dynamic properties of the muscles were determined using the methods and equipment employed previously for work on adult rat muscles (Close, 1964). At the end of each experiment the muscles were fixed at optimal length, macerated in acid and stored in 50 % glycerol (Close, 1964). Fibres were dissected from the muscles and examined under the light microscope at a magnification of × 450. The average sarcomere length was determined for 5–7 fibres from each muscle by counting the number of sarcomeres in a 145 $\mu$ length of fibre in regions every 1·25 mm along the fibre. In two other 1-day-old kittens the twitch:tetanus ratio of SOL was determined for contractions elicited by both indirect and direct stimulation.

## RESULTS

Representative records of isometric contractions of EDL and SOL of a new-born kitten are shown in Fig. 1 together with the force:velocity curves determined from isotonic contractions of the same muscles. Table 1 summarizes the results obtained for three EDL and three SOL muscles from 1- to 2-day-old kittens.

The average isometric twitch contraction time for the EDL muscles was 51 msec recorded at a mean temperature of 34·7° C (34·5–34·8° C). This value for the contraction time ($T_c$) of EDL is shorter than those reported previously for neonatal flexor hallucis longus (FHL) muscle (Buller & Lewis, 1965), but 2–3 times longer than the values obtained for adult

fast muscles (Buller *et al.* 1960; Buller & Lewis, 1965). The mean contraction time for the SOL muscles was 70 msec at 35·5° C. (35·0–36·0° C), which is nearly the same as the times previously reported for both newborn and adult cat soleus muscles (Buller *et al.* 1960). The ratio of half-relaxation time to contraction time $(T_{\frac{1}{2}R}/T_c)$ is 1·0 for EDL and about 1·5 for SOL (Table 1), and in this respect these muscles show little or no
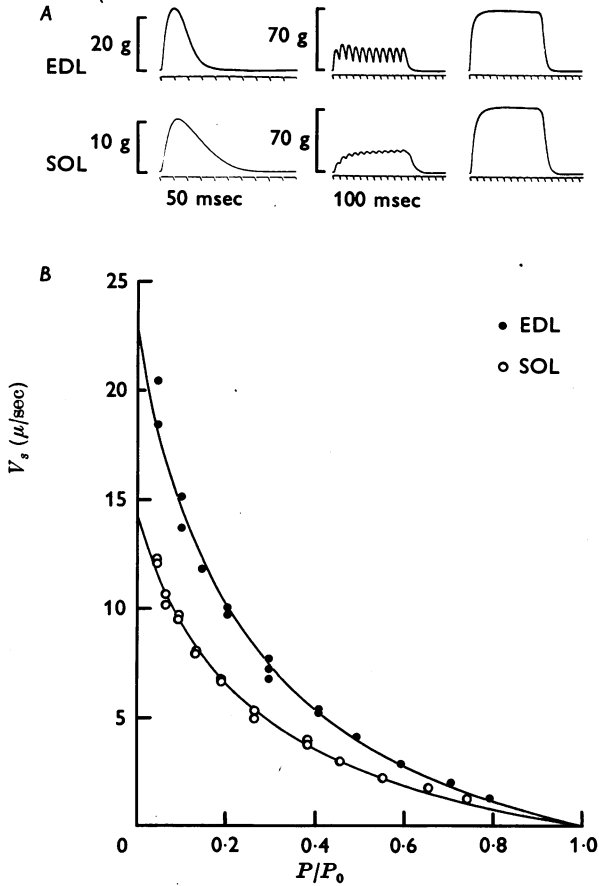


Fig. 1. *A*, Representative records of isometric contractions of extensor digitorum longus (EDL) and soleus (SOL) muscles of a new-born kitten. The records are, from left to right, the isometric twitch and responses to repetitive stimulation at 10 c/s and 100 c/s. *B*, Force–velocity curves for the same extensor digitorum longus (●) and soleus (○) muscles. The speed of shortening of a sarcomere ($V_s$ in $\mu$/sec) is plotted against the isotonic load expressed as a fraction of the maximum isometric tetanic tension; the curves were fitted using Hill's (1938) equation. Muscle weights: EDL = 54 mg, SOL = 56 mg; average muscle fibre length: EDL = 10·1 mm, SOL = 9·5 mm; average sarcomere length: EDL = 3·32 $\mu$, SOL = 3·27 $\mu$.

change during development from birth to maturity. These results show that the isometric twitch of EDL is considerably faster than the twitch of SOL at birth.

The maximum isometric tetanic tension $(P_0)$ was determined at the optimal frequency (Close, 1964), which is about 100 c/s for both EDL and

TABLE 1. Average values for properties of three EDL muscles and three SOL muscles from three new-born kittens. The ranges of values are indicated in parentheses

|  | EDL | SOL |
|---|---|---|
| Muscle wt. (mg) | 53·7 (49–58) | 57 (50–65) |
| Fibre length (mm) | 10·6 (9·75–12·0) | 9·5 (9·27–9·75) |
| Sarcomere length ($\mu$) | 3·25 (3·15–3·32) | 3·24 (3·16–3·28) |
| Maximum isometric tetanic tension (g) | 64 (47–76) | 69·8 (61·0–74·5) |
| Twitch:tetanus ratio | 0·28 (0·19–0·333) | 0·119 (0·103–0·14) |
| Maximum force/area (kg/cm²) | 1·27 (0·94–1·57) | 1·17 (1·12–1·25) |
| Contraction time (msec) | 51 (50–53) | 70 (67–74) |
| Half-relaxation time (msec) | 51 (46–56) | 109 (101–126) |
| Maximum speed of shortening of a sarcomere ($\mu$/sec) | 22·8 (20·0–25·6) | 12·7 (10·0–14·15) |
| $a/P_0$ | 0·265 (0·23–0·295) | 0·315 (0·285–0·375) |

SOL at birth for a tetanus of 1 sec duration. The arrangement of muscle fibres in kitten EDL and SOL is very similar to that described for the corresponding muscles of the rat (Close, 1964) and all the muscle fibres have about the same length. The average cross-sectional area of the muscles calculated from the fibre length and the muscle weight, assuming the density of the muscle to be unity, was $5\cdot3 \times 10^{-2}$ cm² for EDL muscles and $6\cdot0 \times 10^{-2}$ cm² for SOL muscles. The maximum tension developed for unit cross-sectional area of muscle was calculated for each muscle and the average values were 1·27 kg/cm² for EDL and 1·17 kg/cm² for SOL. Thus the intrinsic strength is probably about the same for the two kinds of muscles and is within the usual range (i.e. 1–2 kg/cm²) found for most adult skeletal muscles. The tension developed per gram of muscle is about the same for EDL and SOL at birth and in this respect the results obtained in the present work differ from those reported by Buller & Lewis (1965) for new-born FHL and SOL muscles. The average of the ratios of the isometric twitch tension to the maximum isometric tetanic tension, $P_t/P_0$, was 0·28 for EDL, whereas the average ratio was only 0·119 for the SOL muscles. As the maximum isometric tetanic tension is about the same for EDL and SOL the difference in $P_t/P_0$ is attributable to the difference in peak tension developed in twitches of the two muscles. In two other experiments the $P_t/P_0$ was determined for two neonatal SOL muscles for contractions elicited first by indirect stimulation, *in situ* in one preparation and *in vitro* in the other. The muscles were subsequently curarized *in vitro* ($2\cdot0 \times 10^{-5}$ g of (+)-tubocurarine chloride/ml.) and stimulated

directly with massive platinum electrodes (Mostofsky & Sandow, 1951) using pulses of about 20 V/cm and 0·5 msec duration. $P_0$ and $P_t/P_0$ were nearly the same for the two kinds of stimulation at 35° C and in both preparations $P_t/P_0$ did not exceed 0·1. Consequently the low $P_t/P_0$ for SOL is a property of the muscle itself and is not attributable to failure of some of the muscle fibres to be excited when the muscle is stimulated indirectly by way of the nerve.

The sarcomere length is about the same in neonatal EDL and SOL and the average values obtained were 3·25 $\mu$ for EDL and 3·24 $\mu$ for SOL. The average number of sarcomeres/fibre was estimated for each muscle from the sarcomere length and the fibre length and ranged from 2975 to 3040 sarcomeres/fibre for EDL muscles and from 2830 to 3085 sarcomeres/fibre for the SOL muscles.

The speed of shortening of the musles was determined for different loads and distance–time curves of after-loaded isotonic contractions to repetitive stimulation at the optimal frequency (100 c/s). In order to compare the force:velocity properties of small neonatal muscles and large muscles from older animals it is necessary to express the speed of shortening and the load in terms of unit amounts of contractile material in series and parallel, respectively. For this purpose the speed of shortening of a sarcomere was estimated by dividing the speed of the whole muscle by the average number of sarcomeres per fibre, and the load has been expressed as a fraction of the maximum isometric tetanic tension. Hill's equation (Hill, 1938), $(P+a)V = b(P_0-P)$, in which $P$ = load, $V$ = speed of shortening, $P_0$ = maximum isometric tetanic tension and $a$ and $b$ are constants, was used to fit the force:velocity data obtained for each muscle. The constants describing the force:velocity curves for new-born kitten EDL and SOL muscles are listed in Table 1. In every preparation the EDL was between 1·6 and 2 times faster than SOL with respect to the speed of shortening in isotonic contractions for any given load. The average value for the intrinsic speed of shortening ($V_s^{max}$), i.e. the maximum speed of shortening of a sarcomere when the load is zero, was 22·8 $\mu$/sec for EDL muscles and 12·7 $\mu$/sec for SOL muscles. In the kitten therefore EDL is faster than SOL at birth whether the speed of isotonic shortening or the isometric twitch contraction time is taken as the criterion of speed of contraction.

## DISCUSSION

In order to compare the properties of fast and slow muscles of new-born kittens and adult cats the speed of shortening of sarcomeres of adult cat muscles has been estimated as described previously (Close, 1965b) from the results of work by Fenn & Marsh (1935) and Rosenblueth & Rubio

(1959), except that the sarcomere length was taken to be 2·7 $\mu$ for fast muscle and 3·1 $\mu$ for SOL (Buller & Lewis, 1965). This gives a maximum speed of shortening of sarcomeres of about 31 $\mu$/sec for cat fast muscle and 13 $\mu$/sec for cat SOL. This estimate for adult SOL is about the same as the value obtained for new-born kitten SOL (Table 1). The results show that the intrinsic speed of shortening of SOL does not change during development whereas the intrinsic speed of EDL is increased. In this respect the pattern of change in the speed of cat muscles during differentiation into fast muscles and slow muscles is the same as that for rat and mouse muscles. However, unlike rat and mouse muscles, the speed of shortening of kitten EDL is higher than that of SOL at birth. Nevertheless it is possible that the force:velocity properties of cat EDL and SOL are identical at some earlier stage during foetal development and that the difference between cat and rodent muscles arises because in the kitten the onset of muscle speed differentiation occurs before birth.

The time course of developmental changes in force:velocity properties of cat muscles has not been determined directly in the present work. It is therefore of interest to examine the time course of other changes which may be brought about by changes in the intrinsic speed of shortening. It is known, for example, from the work of Hill (1949) and Jewell & Wilkie (1958) that the time course of tension increase in an isometric tetanus is partly determined by the force:velocity properties of the muscle. The relation between rate of rise of isometric tetanic tension and speed of shortening is not fully understood but the two appear to be approximately proportional in rat muscles (Close, 1964). In this connexion the results of Buller & Lewis (1965) are interesting because they show that the maximum rate of rise of isometric tetanic tension ($\%P_0$/msec) for cat EDL at birth is almost twice as great as the rate for SOL, and that during postnatal development this rate increases in EDL but is not changed in SOL. This pattern of change is similar to that for changes in the maximum speed of shortening of sarcomeres ($V_s^{max}$). A further observation indicating a correspondence between $\%P_0$/msec and $V_s^{max}$ is seen in the relation between each one of these properties and the isometric twitch contraction time. It has been shown for a number of muscles that there is a hyperbolic relation between $V_s^{max}$ and $T_c$ (Close, 1964, 1965b) and a similar inverse relation between $\%P_0$/msec and $T_c$ is evident in Fig. 7 of the paper by Buller & Lewis (1965). It seems likely therefore that in cat muscles $\%P_0$/msec and $V_s^{max}$ are approximately proportional and that developmental changes in $V_s^{max}$ largely determine the changes in the time course of tension development in isometric tetanic contractions. The data presented by Buller & Lewis (1965) on the time course of changes in $\%P_0$/msec (Fig. 4$B$, 1965) show that fast muscles attain adult values for $\%P_0$/msec within

5–6 weeks after birth and it is likely that the changes in force:velocity properties of EDL follow a similar time course.

The developmental changes in isometric twitch contraction time of rat muscles have been accounted for in terms of changes in both $V_s^{max}$ and $P_t/P_0$ (Close, 1964) and it is of interest to ascertain whether the changes in the cat muscles can be explained similarly. In rat muscles a change in $P_t/P_0$ leads to an approximately proportional change in $T_c$ and a change in $V_s^{max}$ brings about an inversely proportional change in $T_c$. For newborn cat fast muscle $P_t/P_0$ is approximately 0·3 (Table 1 above; Buller & Lewis, 1965) and $V_s^{max}$ is 22·8 $\mu$/sec, whereas for adult fast muscle $P_t/P_0$ is about 0·24 (Buller & Lewis, 1965) and $V_s^{max}$ is about 31 $\mu$/sec (above). These changes in $P_t/P_0$ and $V_s^{max}$ may be expected to alter the contraction time from about 51 msec at birth to about 30 msec for adult muscles. This estimated time for contraction of adult fast muscle is larger than the values reported by Buller & Lewis (1965) but corresponds fairly well with the contraction times of about 27 msec recorded by Buller *et al.* (1960) for several fast muscles. The pattern of change in various properties of cat fast muscles therefore resembles closely the patterns described previously for rat and mouse muscles.

The development of cat SOL is similar to that of rat and mouse SOL muscles in that there are no changes in $V_s^{max}$, but it differs from the others with respect to changes in $P_t/P_0$ and $T_c$. In rodent SOL muscles there are proportional decreases in $P_t/P_0$ and $T_c$ during the first few weeks after birth, whereas in cat SOL there is little or no change in $T_c$ but there is a twofold increase in $P_t/P_0$ from about 0·119 (Table 1) at birth to about 0·26 within 2 weeks. It is unlikely that this change is due to a decrease in the relative compliance of the passive series elastic elements because both % $P_0$/msec and $V_s^{max}$ remain unchanged throughout development. The possibility that the low $P_t/P_0$ of SOL at birth results from failure of some muscle fibres to be excited when the muscle is stimulated indirectly has been excluded because the responses of the muscle to direct and indirect stimulation are the same. An alternative explanation is that at birth the individual muscle fibres of cat SOL are incompletely activated either because the muscle fibre action potential fails to activate all the myofibrils and cross bridges or because the tension-generating sites fail to develop maximum tension in the twitch.

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