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THE RELATION BETWEEN INTRINSIC SPEED OF SHORTENING AND DURATION OF THE ACTIVE STATE OF MUSCLE

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The form of the isometric-twitch contraction of skeletal muscle is dependent upon the properties of the series-elastic elements, the time course of the active state and the force:velocity properties of the contractile material (Hill, 1938, 1949*a*; Macpherson, 1953; Ritchie, 1954*b*; Macpherson & Wilkie, 1954; Jewell & Wilkie, 1958). It has been demonstrated that the intrinsic properties of the contractile material are independent of certain changes in the time course of the active state. For example, nitrate ions prolong the active state (cf. reverse of Fig. 1*B*), thereby increasing both the isometric-twitch contraction time and the twitch:tetanus ratio, but have no effect upon the force:velocity properties (Hill & Macpherson, 1954; Ritchie, 1954*c*; Kahn & Sandow, 1950, 1955). There is, however, no information concerning the changes which would result from alteration of the intrinsic speed of shortening of the contractile material. Goffart & Ritchie (1952) and Ritchie (1954*c*) have commented on the possibility that a decrease in intrinsic speed should increase the isometric-twitch contraction time and decrease the twitch:tetanus ratio, there being presumably no concomitant change in the time course of decay of the active state. This effect would be similar to that resulting from an increase in compliance of the series-elastic elements (cf. Fig. 1 below and Hill, 1951*a*). In contrast, Close (1964) has suggested that the duration of the active state is dependent upon the intrinsic speed of shortening of the contractile material Accordingly a change in the time course of the active state which is dependent upon alteration of the force:velocity properties would lead to a change in the isometric-twitch contraction time which is inversely proportional to the change in the speed of shortening, but there would be no change in the twitch:tetanus ratio because the same mechanical events would occur on an altered time scale (Fig. 1*C*).

These opposing viewpoints could be tested if it were possible to alter the force:velocity properties of the contractile material. At the present time

there is available no chemical agent which is known to have such a specific effect when applied to intact muscle fibres. However, the intrinsic speed of shortening of sarcomeres (defined below) may be obtained for some muscles by dividing the speed of the whole muscle by the number of sarcomeres in each muscle fibre. In this way it has been shown that the intrinsic speed of shortening not only differs in different muscles but is altered during development in some muscles (Close, 1964, 1965*a*). In this investigation an attempt has been made to determine the relation between the duration of the active state and the intrinsic speed of shortening by comparing the properties of rat and mouse muscles at different stages of development and the properties of neonatal rat extensor digitorum longus muscles at different temperatures. It will be shown that the isometric-twitch contraction time is inversely proportional to the intrinsic speed of shortening and that the twitch:tetanus ratio is independent of changes in the intrinsic speed.

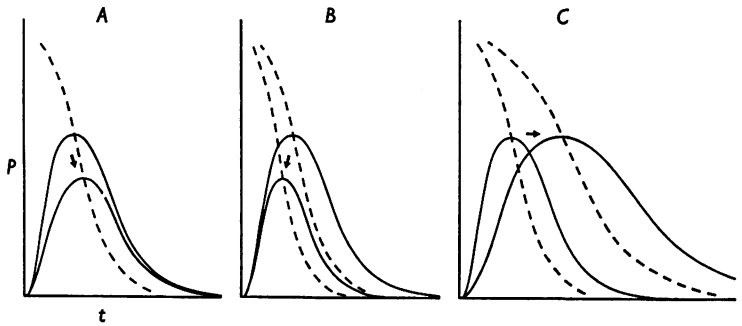

Fig. 1. Diagrams representing various changes in the form of the isometric twitch (———) and the time course of decay of the active state (– – – –). The arrows indicate the direction of change in the peak of the twitch. *A*. A decrease in series compliance as described by Hill (1951*a*). *B*. A decrease in the duration of the active state such as that suggested to account for the developmental changes of rat soleus muscles. Prolongation of the duration of the active state by nitrate ions gives the reverse effect as described in the text above. *C*. A decrease in the intrinsic speed of shortening to one half of the original value. The tension (P) and time (t) are represented on the ordinates and abscissae, respectively.

METHODS

The methods used for determining the developmental changes in the dynamic properties of rat and mouse extensor digitorum longus (EDL) and soleus (SOL) muscles have been described in detail (Close, 1964, 1965*a*). The same methods and equipment have been employed in the present work to determine the effects of temperature on contractions of neonatal rat EDL muscles *in vitro*. These muscles were examined within 12 hr after birth and the duration of each experiment was from 4 to 6 hr. Each muscle was connected vertically to an isotonic lever (equivalent mass = 12 mg) through a straight steel-wire connexion (mass = 10 mg) tied directly to one tendon. The other tendon was firmly clamped and the whole muscle was bathed in about 500 ml. of Ringer's solution (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 aerated with a mixture containing 95 % O_2 and 5 % CO_2. Curare (1×10^{-2} g or $2{\cdot}5 \times 10^{-3}$ g/l.) was added to the bath fluid in all but one of the experiments and there was no difference in the results as a consequence. The isotonic lever could be connected to a mechano-electronic transducer (RCA 5734) for the measurement of tension in isometric responses and the whole apparatus could be raised or lowered to alter the length of the muscle. In these conditions the compliance of the recording equipment was less than 5×10^{-4} cm/g. The optimal length was determined for each muscle and the speed of shortening in after-loaded isotonic tetanic contractions was measured in the usual way (Close, 1964). The muscle was stimulated transversely by maximal, or supramaximal, bipolar, square pulses of 0·5 msec duration through two platinum-wire electrodes. The optimal frequency for repetitive stimulation was 75 c/s at 35° C and 35 c/s at 25° C. During the course of an experiment the maximum isometric tetanic tension usually decreased to about 97 % (94–100 %) of the original value but the twitch tension did not change.

The same methods as described previously (Close, 1964) were used for determining the average number of sarcomeres/fibre and for fitting Hill's equation (Hill, 1938; $(P+a)V = b(P_o-P)$, where P = load, V = speed of shortening, P_o = maximum isometric tetanic tension, and a and b are constants) to the force:velocity measurements. The optimal length (L_o), optimal frequency for repetitive stimulation (f_o), contraction time (T_c), and the maximum isometric tetanic (P_o) and twitch (P_t) tensions were defined previously (Close, 1964). Throughout this work the speed of shortening is expressed in terms of the speed of shortening of one sarcomere (V_s) in μ/sec, obtained by dividing the speed of the whole muscle by the average number of sarcomeres/fibre. The load is expressed as the percentage of the maximum load equivalent to P_o.

The *intrinsic speed of shortening* is defined as the maximum speed of shortening of a sarcomere at optimal length. The shape of the force:velocity curve, as indicated by the values for a/P_o from Hill's equation, is virtually identical in all of the muscles which have been examined (Close, 1964, Table 1; Close, 1965*a*, Table 1; Table 1 below). For comparative purposes therefore the intrinsic speed may be taken to be the speed of shortening of a sarcomere for a load equal to any given fraction of the maximum load.

RESULTS

Effect of temperature on contraction of rat muscle

The effect of temperature changes on isometric contractions of new-born rat EDL *in vitro* is essentially the same as the effects described by South (1961) for hamster diaphragm. The optimal temperature for maximal tension development in twitch and tetanus is about 30° C and there was little variation between 25 and 35° C. The mean values for the maximum isometric tetanic tension (P_o) were the same for contractions at 25 and 35° C and this was 95 % of the value for P_o at 30° C.

The data presented in Table 1 are the mean values obtained for three muscles at 25° C and another three muscles at 35° C. In each of these groups the average size of the muscles was about the same as shown by the values for weight and the number of sarcomeres per fibre. The effect of load on the speed of shortening, as indicated by the values for a/P_o, is virtually the same at the two temperatures. Furthermore, the tension developed in isometric contractions, and consequently the twitch:tetanus

ratio, is nearly the same at 25 and 35° C. In contrast the speed of shortening and the isometric-twitch contraction time are greatly altered by this change in temperature.

TABLE 1. Properties of neonatal rat EDL muscle *in vitro* at different temperatures: mean values and the ranges of values for three muscles at 25° C and for three muscles at 35° C

	25° C	35° C
Muscle weight (mg)	1·72 (1·7–1·8)	1·62 (1.35–1·8)
Sarcomeres/fibre	1056 (938–1188)	1107 (1071–1169)
Maximum isometric tetanic tension (g)	2·52 (2·32–2·65)	2·52 (2·29–2·95)
Twitch: tetanus ratio	0·5 (0·48–0·52)	0·54 (0·485–0·604)
a/P_o (Hill's equation)	0·184 (0·14–0·23)	0·22 (0·194–0·256)
Maximum speed of shortening of one sarcomere (μ/sec)	14·4 (13·13–15·7)	24·2 (22·45–27·9)
Contraction time (msec)	101 (97·0–104·5	56 (50·5–59·0)

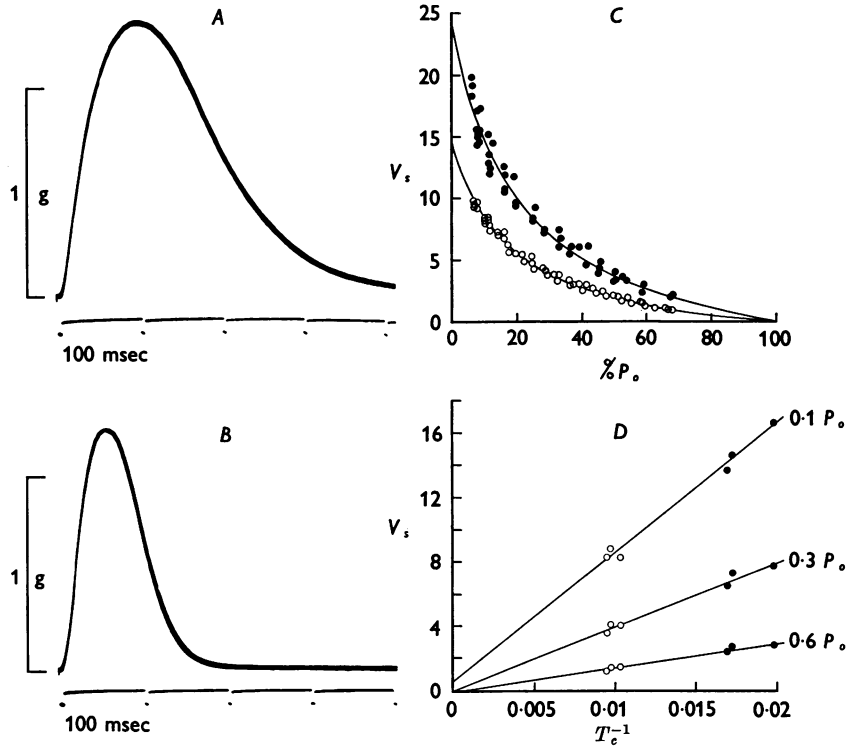

Fig. 2. The effect of temperature on isometric-twitch contractions (*A*, *B*) and the force: velocity relation (*C*) of neonatal rat EDL muscles *in vitro*. *A* and *B*. Records of the isometric twitch of one muscle at 25° C (*A*) and of another muscle at 35° C (*B*). *C*. The speed of shortening per sarcomere (V_s, in μ/sec) plotted against the load expressed as a percentage of the maximum load equivalent to P_o (% P_o), for three muscles at 25° C (open circles) and another three muscles at 35° C (filled circles). *D*. The speed of shortening (V_s) calculated from Hill's equation for individual muscles plotted against the reciprocal of the isometric-twitch contraction time (T_c, in msec).

Figures 2*A* and *B* are representative records of the isometric twitches of two muscles at 25 and 35° C. Figure 2*C* shows the force:velocity curves obtained for three muscles at 25° C and another three muscles at 35° C. In Fig. 2*D* the speed of isotonic shortening (V_s) for three different loads is plotted against the reciprocal of the isometric-twitch contraction time for each of the six muscles which were examined. The lines drawn in this graph were fitted by the method of least squares for the regression of V_s on $1/T_c$. The equations describing these lines are given below together with the standard deviations of V_s from the regression lines in parentheses.

When $P = 0{\cdot}1\ P_o$, $V_s = 807/T_c + 0{\cdot}57$ $(\pm 0{\cdot}511)$;

$P = 0{\cdot}3\ P_o$, $V_s = 401/T_c - 0{\cdot}095$ $(\pm 0{\cdot}386)$;

$P = 0{\cdot}6\ P_o$, $V_s = 149/T_c - 0{\cdot}114$ $(\pm 0{\cdot}318)$.

The correlation coefficients for these data were 0·995, 0·99 and 0·976 when P was $0{\cdot}1P_o$, $0{\cdot}3P_o$ and $0{\cdot}6P_o$, respectively, thus indicating that the regression lines for $1/T_c$ on V_s lie close to those drawn in Fig. 2*D*. The values for the intercepts on the ordinates in the above equations are approximately the same or less than the standard deviation of the observed values of V_s from the regression line. It may be concluded therefore that failure of the lines to pass through the origin is not significant and that in this example there is a hyperbolic relation between the intrinsic speed of shortening (V_s) and the isometric-twitch contraction time (T_c). In other words for a given load $V_s \times T_c = k$ where k is a constant. It should be pointed out that the speed of shortening/sarcomere was consistently greater in this series of measurements than those reported previously (Close, 1964; see Table 2 below); the reason for this difference is not known.

A temperature coefficient near unity has been reported for the maximum tension and the twitch:tetanus ratio in amphibian muscle, 0–20° C (Jewell & Wilkie, 1958), locust flight muscles, 25–45° C (Neville & Weis-Fogh, 1963) and for hamster diaphragm from about 24° C to 38° C (South, 1961). In all of these muscles the rate of contraction is markedly affected by changes in temperature.

Developmental changes in force:velocity properties

The pattern of post-natal development of fast and slow limb muscles is similar in the rat and the mouse (Close, 1964, 1965*a*). Neonatal EDL, neonatal SOL and juvenile SOL muscles have almost identical force:velocity properties (Table 2, Fig. 3*C*, *D*), whereas the speed of shortening of EDL increases two to three times during development (Table 2, Fig. 3*C*, *D*). The isometric-twitch contraction time is about the same in EDL and SOL muscles at birth; thereafter it decreases for both muscles but to a greater extent in the fast, EDL, muscle in proportion to its increased speed of shortening (Fig. 3*A* and *B*).

These changes are complicated by progressive decreases in values for k and the twitch:tetanus ratio, which occur concurrently and to about the same extent in both muscles. Figure 4*A* shows the relation between P_t/P_o and k (from $k = T_c \times V_s$ for $P = 0{\cdot}1P_o$) for rat and mouse EDL and SOL muscles at different stages of development. The correlation coefficient for these two sets of measurements is 0·956 and the line ($P_t/P_o = 0{\cdot}7k - 0{\cdot}004$) has been fitted for the regression of P_t/P_o on k. The muscles represented in the graph had a fivefold range of speeds of shortening. Figure 4*A* shows that whereas there are large changes in k and

TABLE 2. The speed of shortening (V_s), isometric-twitch contraction time (T_c), k ($k = T_c$ in seconds $\times V_s$ for $P = 0{\cdot}1P_o$) and twitch:tetanus ratio (P_t/P_o) of rat and mouse extensor digitorum longus (EDL) and soleus (SOL) muscles at different stages of development. All measurements were made at 35–36° C.

	Age (days)	V_s, ($P = 0{\cdot}1P_o$) (μ/sec)	T_c (msec)	k (μ)	P_t/P_o
Rat SOL	0	12·15	65·0	0·790	0·64
Rat EDL	0	11·1	56·5	0·627	0·53
Rat SOL	10	9·6	43·0	0·413	0·36
Rat EDL	10	20·6	24·5	0·505	0·29
Rat SOL	35	11·7	28·5	0·333	0·21
Rat EDL	35	30·4	10·75	0·327	0·18
Rat SOL	100	10·15	36·0	0·365	0·19
Rat EDL	100	27·0	12·5	0·338	0·246
Mouse SOL	0	19·77	51·0	1·005	0·71
Mouse EDL	0	19·23	41·5	0·80	0·59
Mouse SOL	30	21·0	14·5	0·305	0·23
Mouse EDL	30	39·2	8·0	0·314	0·24

P_t/P_o in SOL muscles during development with no significant change in the force:velocity properties, EDL muscles undergo a comparable change in k and P_t/P_o even though there is a twofold to threefold increase in the speed of shortening during development. Figure 4*A* indicates that P_t/P_o is directly proportional to ($T_c \times V_s$) and it further shows that this relation is independent of the speed of shortening (V_s). Consequently, within the range of the measurements, the isometric-twitch contraction time (T_c) has approximately a directly proportional relation to P_t/P_o for any particular intrinsic speed of shortening. This point is illustrated in fact by the approximately proportional changes in T_c and P_t/P_o in SOL muscles during development (Table 2) with virtually no change in the force:velocity properties (Fig. 3*C*, *D*). Proportional increases in twitch height and contraction time are evident in records of isometric twitches of frog sartorius muscles in the presence of nitrate ions (e.g. Ritchie, 1954*c*, Fig. 3; Close, 1962, Fig. 4); a similar observation has been made by Isaacson & Sandow (1963) for twitches potentiated by zinc ions.

Changes in isometric-twitch contraction time which are associated with

developmental changes in the intrinsic speed of shortening may be demonstrated by taking into account the changes which result from alteration of the twitch:tetanus ratio. As a first approximation it may be assumed that T_c is directly proportional to P_t/P_o (see above) for a

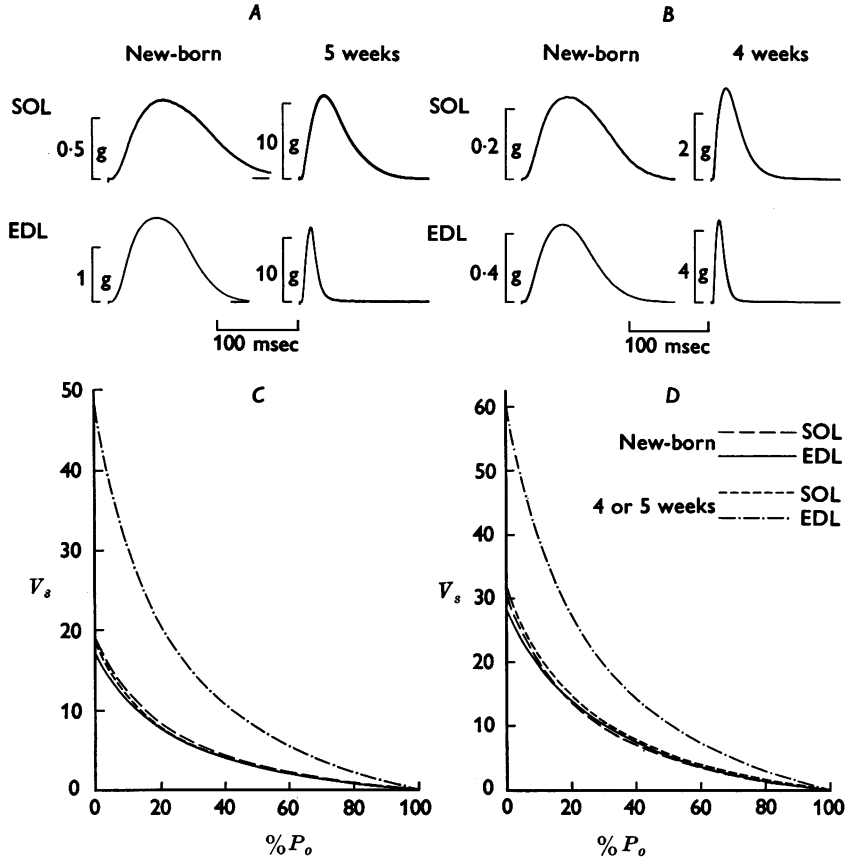

Fig. 3. Summary of the developmental changes in the form of isometric twitches and the force:velocity properties of rat and mouse muscles. *A*. Records of isometric twitches of EDL and SOL muscles from new-born and 5-week-old rats. *B*. Records of isometric twitches of EDL and SOL muscles from new-born and 4-week-old mice. *C* and *D*. Curves drawn from Hill's equation describing the force:velocity properties of new-born and 5-week-old rat muscles (*C*) (from Close, 1964) and new-born and 4-week-old mouse muscles (*D*) (from Close, 1965*a*). All measurements were made at 35–36° C.

given intrinsic speed. Values for contraction time for any particular P_t/P_o may then be calculated for each muscle from the observed values. The result of such a calculation is shown in Fig. 4*B* in which the speed of shortening is plotted against the reciprocal of the contraction time

(T_c^*) calculated for $P_t/P_o = 0{\cdot}5$. These values for T_c^* were obtained from the data in Table 2 using the equation

$$T_c^* = \frac{T_c \times 0{\cdot}5}{P_t/P_o}.$$

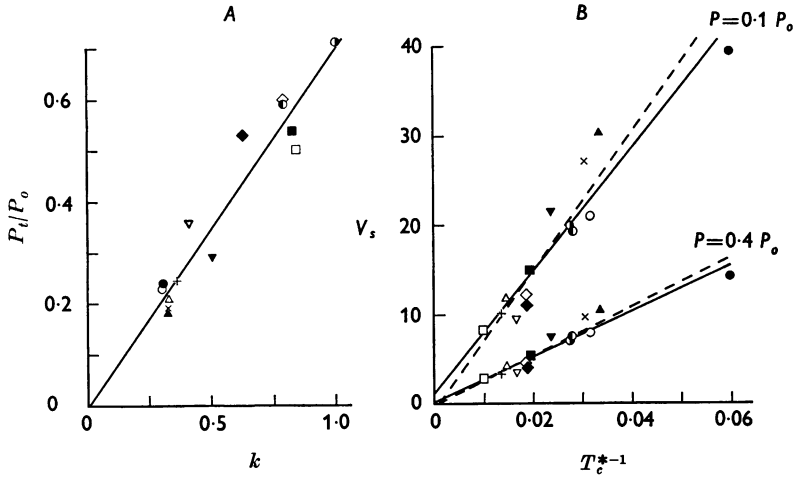

Fig. 4. *A*. The relation between the twitch: tetanus ratio (P_t/P_o) and k ($k = T_c \times V_s$ for $P = 0{\cdot}1P_o$, from Tables 1 and 2 above) for rat and mouse muscles at different stages of development. *B*. The relation between the speed of shortening, V_s for $P = 0{\cdot}1P_o$ (upper set) and $P = 0{\cdot}4P_o$ (lower set), and the contraction time (T_c^*). T_c^* was calculated as described in the text for $P_t/P_o = 0{\cdot}5$. In both *A* and *B* the points are for new-born mouse EDL ◐ and SOL ◑, 30-day-old mouse EDL ● and SOL ○, new-born rat EDL ◆ and SOL ◇, 10-day-old rat EDL ▼ and SOL ▽, 35-day-old rat EDL ▲ and SOL △, and 100-day-old rat EDL × and SOL +; all of these measurements were made at 35–36° C. Values for neonatal rat EDL *in vitro* at 35° C ■ and 25° C □ have been included.

The lines fitted to the points in Fig. 4*B* are for the regression of V_s on $1/T_c^*$ and vice versa. The equations describing these lines are given below together with the standard deviation from the regression lines in parentheses. When

$$P = 0{\cdot}1P_o, \quad V_s = 685/T_c^* + 1{\cdot}21 \ (\pm 3{\cdot}04),$$
$$1/T_c^* = 0{\cdot}0013V_s + 0{\cdot}00095 \ (\pm 0{\cdot}00425)$$

and the correlation coefficient = 0·952. When

$$P = 0{\cdot}4P_o, \quad V_s = 255/T_c^* + 0{\cdot}18 \ (\pm 1{\cdot}015),$$
$$1/T_c^* = 0{\cdot}00358V_s + 0{\cdot}00145 \ (\pm 0{\cdot}0038)$$

and the correlation coefficient = 0·962. There is, therefore, a linear association between the two sets of values. In all these equations the value for the intercept is much less than the standard deviation from the

regression line and it may be concluded that failure of the lines to pass through the origin is not significant. In other words, part of the change in contraction time of EDL muscles is attributable to the change in Pt/P_o, the remainder is inversely proportional to the developmental change in the intrinsic speed of shortening.

The average values for P_t/P_o for juvenile mouse (30 days) and rat (35 days) EDL and SOL muscles fall within the range 0·18–0·24 and the corresponding values for k range from 0·305 to 0·333 μ/sarcomere (Table 2). The relation between V_s and T_c for these muscles may therefore be demonstrated without having to make allowances for differences in P_t/P_o. In this case the regression lines relating V_s and $1/T_c$ are described by the following equations; the standard deviation from regression is given in parentheses in each case. When

$$P = 0{\cdot}1P_o, \quad V_s = 319/T_c + 0{\cdot}06\ (\pm 1{\cdot}073);$$

$$P = 0{\cdot}3P_o, \quad V_s = 159/T_c - 0{\cdot}08\ (\pm 0{\cdot}213);$$

$$P = 0{\cdot}6P_o, \quad V_s = 59{\cdot}5T_c - 0{\cdot}07\ (\pm 0{\cdot}082).$$

The correlation coefficient for each of these three groups of measurements was between 0·997 and 0·999. The value for V_s at the intercept is less than the standard deviation of the observed values of V_s from the regression line. It may be concluded therefore that the relation between V_s and T_c in these muscles is accurately described by a hyperbola. The principal differences between these juvenile fast and slow muscles may be attributed to differences in their intrinsic speeds of shortening.

DISCUSSION

All the results described above indicate the existence of a hyperbolic relation between the intrinsic speed of shortening and the isometric-twitch contraction time. In no instance was there a change in the twitch: tetanus ratio attributable to a change in speed or any other indication that the time course of the active state is independent of changes in the intrinsic speed of shortening.

Changes in force: velocity properties are also known to occur in mammalian muscles following cross-union of the motor nerves to fast and slow muscles (Close, 1965*b*) and in amphibian muscle bathed in hypertonic solutions (Howarth, 1958). The effects of cross-union of nerves to mammalian muscles have been attributed to changes in the intrinsic speed of shortening, there being inversely proportional changes in the intrinsic speed of shortening and twitch contraction time with no net change in the twitch: tetanus ratio. Howarth's observation that the duration of the active state of frog muscle is only slightly increased in hypertonic solutions

when the speed of shortening is greatly decreased suggests that a simple inverse proportionality does not exist in this instance. However, it is not known whether the decrease in speed of shortening in hypertonic solutions involves a change in the intrinsic properties of the contractile material or whether it is due merely to a mechanical restraint or to some other effect analogous to the extrinsic control of speed by the load.

The question arises whether the isometric responses of all muscles differ principally as a result of differences in their intrinsic speeds of shortening. Table 3 lists the properties of various skeletal muscles for comparison with those of rat and mouse limb muscles. Average values for the maximum speed of shortening per sarcomere (V_s in μ/sec) were calculated from values for speed in muscle lengths/sec assuming a sarcomere length of 2·5 μ for rat diaphragm, cat, man and toad muscles, 2·1 μ for frog sartorius muscle (Gordon, Huxley & Julian, 1964) and 4·0 μ (Weis-Fogh, 1956) for locust flight muscle. In the cat skeletal muscles referred to in Table 3 the individual muscle fibres do not run from one end of the muscle to the other. However all the muscle fibres have about the same length and are arranged in parallel, extending from one tendon to the other in much the same way as the fibres in rat EDL and SOL (Close, 1964). Values for the intrinsic speed of shortening of sarcomeres of cat muscles were obtained from the equation

$$V_s = \frac{V \times S_L}{M_L \times R}$$

in which V_s is the speed of shortening of a sarcomere in μ/sec, V and M_L are respectively the maximum speed of shortening of the whole muscle (μ/sec) and the muscle length (μ) given by Fenn & Marsh (1935) and Rosenblueth & Rubio (1959), S_L is the sarcomere length (assumed to be 2·5 μ) and R is the ratio of muscle fibre length (F_L) to muscle length (M_L), i.e. $R = F_L/M_L$. The ratio R was determined from dissections in this laboratory to be 0·4 for cat soleus muscles and 0·35 for rectus femoris and vastus lateralis muscles of the quadriceps.

The data in Table 3, some of which have been plotted in Fig. 5, show that there is a fairly linear association between the intrinsic speed of shortening and the isometric-twitch contraction time irrespective of differences in twitch:tetanus ratios. In this instance it is not possible to establish exact relations for V_s, T_c, P_t/P_o and k because the influence of a number of factors upon the form of the isometric twitch cannot be assessed. For example, differences in the relation between V_s and T_c, and consequently between k and P_t/P_o may be associated with differences in properties of the series-elastic elements and the time course of decay of the active state (Fig. 1*A*, *B*).

The relation between k and P_t/P_o is not one of direct proportionality in

Table 3 as it is for mouse and rat muscles (Table 2 and Fig. 4*A*). For example, the values for k for rat SOL and locust flight muscles are about the same whereas their twitch:tetanus ratios differ by a factor of three.

TABLE 3. Average values for the maximum speed of shortening per sarcomere (V_s in μ/sec), the isometric-twitch contraction time (T_c in msec) and the twitch:tetanus ratio (P_t/P_o). Values for k were calculated from $k = T_c$ in seconds $\times V_s$ for $P = 0$. The numbers in parentheses are for the references listed below

Muscle	Temp. (° C)	V_s ($P = 0$)	T_c (msec)	P_t/P_o	k (μ)
Mouse EDL	35–36	60 (9)	8·0 (Table 2 above)	0·24 (Table 2 above)	0·480
Rat EDL	35–36	43 (8)	12·5 (8)	0·19 (8)	0·537
Mouse SOL	35–36	32 (9)	14·5 (Table 2 above)	0·23 (Table 2 above)	0·464
Locust flight	30	30 (5)	23 (5)	0·5 (23)	0·690
Rat diaphragm	37	27·5 (25)	18 (14, 11)	0·14 (14)	0·495
Cat fast	36	26 (12)	27 (6)	0·29 (10)	0·702
Rat SOL	35–36	18 (8)	36 (8)	0·25 (8)	0·648
Frog sartorius	20–22	12·6 (15)	38 (4, 13, 30)	0·38 (30)	0·479
Cat SOL	36	10·5 (29)	70 (6)	0·26 (10)	0·735
Locust flight	11	6 (5)	100 (5)	0·77 (5)	0·600
Man	37	6 (31)	110 (24)	—	0·660
Frog sartorius	0–2	3·3 (2, 3, 15, 21, 22, 28)	220 (7, 16, 19, 26, 27)	0·75 (2, 18, 26, 27)	0·726
Toad sartorius	0	2·1 (16)	430 (1, 17, 20)	0·62 (16)	0·903

(1) Abbott, Aubert & Hill, 1951. (2) Abbott & Lowy, 1953. (3) Abbott & Wilkie, 1953. (4) Brown & Sichel, 1936. (5) Buchthal, Weis-Fogh & Rosenfalck, 1957. (6) Buller, Eccles & Eccles, 1960. (7) Close, 1962. (8) Close, 1964. (9) Close, 1965. (10) Cooper & Eccles, 1930. (11) Creese, Hashish & Scholes, 1958. (12) Fenn & Marsh, 1935. (13) Gilson, Schoepfle & Walker, 1947. (14) Goffart & Ritchie, 1952. (15) Hill, 1938. (16) Hill, 1949*a*. (17) Hill, 1949*b*. (18) Hill, 1951*b*. (19) Hill, 1953. (20) Hill & Howarth, 1959. (21) Jewell & Wilkie, 1958. (22) Katz, 1939. (23) Neville & Weis-Fogh, 1963. (24) Prosser & Brown, 1961. (25) Ritchie, 1954*a*. (26) Ritchie, 1954*c*. (27) Ritchie & Wilkie, 1955. (28) Ritchie & Wilkie, 1958. (29) Rosenblueth & Rubio, 1959. (30) Sandow, 1947. (31) Wilkie, 1954.

Furthermore, k for frog sartorius at 0° C is only about one half of the value which would be expected (Fig. 4*A*) for rat and mouse muscles having the same twitch:tetanus ratio. It is to be expected that the relation between k and P_t/P_o is dependent not only upon the duration of the active state of myofibrils (Fig. 1*B*) but also upon the degree of activation of sarcomeres, i.e. the number of parallel myofibrils which are activated during a twitch compared with the number operating during a tetanus. Partial activation of this kind would lead to a smaller twitch:tetanus ratio but would not alter the time course of the twitch or the value for k.

It is difficult to assess the degree of activation of sarcomeres for any muscle. Two experiments have been described which suggest that frog sartorius muscle is fully activated at about 40 msec after the stimulus at 0° C (Hill, 1949*a*; Close, 1962) though Jewell & Wilkie (1958) have reported that these muscles might not always be completely activated during a twitch. Jewell & Wilkie found that P_t/P_o ranged from 0·85 to 0·92 at 0° C (cited by Hill, 1958) with a temperature coefficient near unity. In view of this the low values for P_t/P_o

reported by Sandow (1947) and Hill (1951*b*) for maximally stimulated frog sartorius muscles at room temperature may have been due in part to incomplete activation of sarcomeres involving only a fraction of the total amount of contractile material. Post-tetanic potentiation of the isometric-twitch response of mammalian fast muscle with virtually no change in the contraction time (Brown & von Euler, 1938) may be another example of partial activation of sarcomeres.

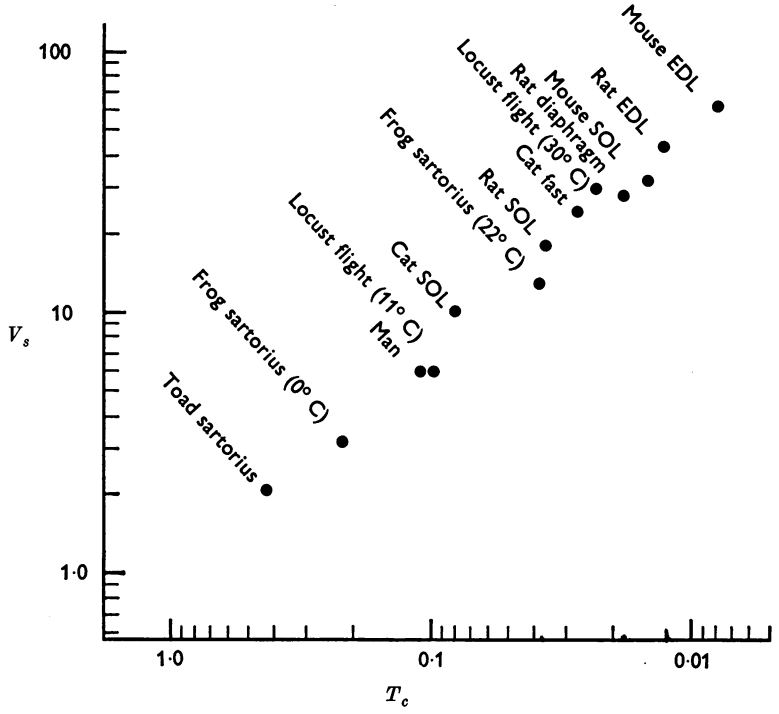

Fig. 5. The relation between the maximum speed of shortening of a sarcomere (V_s) in μ/sec and the contraction time T_c (seconds). The values were obtained from Table 3 and both scales are logarithmic.

Some muscles may show differences in time course of decay of the active state which are not due to differences in the intrinsic speed of shortening (Fig. 1*B*). The extent to which this may account for some of the differences between the muscles listed in Table 3 is not known. It has been suggested (Close, 1964, and above) that this kind of change leads to all or part of the decrease in k and P_t/P_o during development in rat and mouse SOL and EDL muscles. Furthermore, it has been shown above that at any stage of development the differences in the contraction time of fast and slow rat and mouse muscles are attributable partly to differences in P_t/P_o and partly to differences in the force:velocity properties. For this reason the data for new-born rat and mouse muscles have not been included in Table 3 or Fig. 5.

If the differences in the twitch:tetanus ratio (Table 3) were due entirely to differences in the degree of activation of sarcomeres, the relation between V_s and T_c in Fig. 5 would be unaffected. On the other hand, if these differences in the size of the twitch were due to differences in the duration of the active state of myofibrils (cf. Fig. 1*B*), and if allowances were made accordingly, the slope of the line joining the points in Fig. 5 would be increased. In either case the speed of shortening and the contraction time would be approximately inversely proportional. It may be concluded that among different muscles there may be differences both in the relation between V_s and the duration of the active state and in the degree of activation of sarcomeres in a twitch, but the main difference in twitch-contraction times appears to be of the kind which is associated with differences in the intrinsic speed of shortening (Fig. 1*C*).

The hyperbolic relation between intrinsic speed and contraction time may be demonstrated for different muscles which have the same sarcomere length whether the speed of shortening of the sarcomere is expressed as sarcomere lengths/sec or μ/sec. However, in comparing muscles which differ in sarcomere length (e.g. frog sartorius 2·1 μ, locust flight muscle 4·0 μ) it would be necessary to express the speed in one unit or the other depending upon whether the contractile filaments fold or slide during contraction. The values for k for frog sartorius (0° C) and locust flight (11° C) muscles are about the same when calculated from the intrinsic speed of shortening in μ/sec in accordance with the view that contractile filaments slide, rather than fold, during contraction. In contrast the values for k differ by a factor of about two when the speeds of these two muscles are expressed in sarcomere lengths/sec, as should be done to describe the dynamic properties of a system which shortens by folding of filaments. Thus the hyperbolic relation between intrinsic speed and contraction time can be demonstrated in these muscles which differ in sarcomere length but only in terms of the sliding-filament mechanism of contraction.

In the equation $V_s \times T_c = k$, the proportionality factor k has the dimensions of length (μ/sarcomere). Throughout this work it has been convenient to express the relation between V_s and T_c in this way but it should be pointed out that in calculating the values for k given above (Tables 2 and 3, Fig. 4*A*) no allowance has been made for the effects of decay of the active state or changes in length, both of which modify V_s during twitch contractions. Consequently these values for k are only proportional to the actual shortening of the contractile element in either isometric or isotonic twitch contractions. Nevertheless, k is constant irrespective of alterations in the intrinsic speed of shortening and is a useful indicator of this kind of change.

The factor k being proportional to the amount of shortening which the sarcomere undergoes in particular conditions is also proportional to the amount of work done following a single stimulus. In connexion with the work done in the isometric contractions described above it may be noted that the properties of the series–elastic elements of neonatal rat EDL muscle are probably little affected by a 10° C change in temperature (cf. Jewell & Wilkie, 1958) and that indirect evidence suggests that the relative series compliance is about the same in fast and slow muscles of the rat at all stages of development (Close, 1964). Consequently the amount of work done in stretching the elastic elements during an isometric-twitch contraction should not be altered by changes in the intrinsic speed of shortening. The same result has been obtained from experiments on rat cardiac ventricular muscle *in vitro* in which, for a given load, the same amount of work is done in isotonic twitches at 20 and 30° C even though the temperature coefficient for the rate of shortening was equal to 2·6 (unpublished observation).

Concerning the nature of the mechanism underlying the hyperbolic relation between V_s and T_c it is important to note that V_s may be proportional to the rate of a chemical reaction. In this connexion, Bárány, Bárány, Reckard & Volpe (1965) have found that the rate of hydrolysis of adenosinetriphosphate by myosin is 2 to 3 times higher for rabbit EDL than for rabbit SOL. This ratio of rates of hydrolysis is about the same as the ratio of the contraction speeds for mammalian fast and slow muscles. In addition there is an increase in the activity of myosin adenosinetriphosphatase in rat muscles during development (De Villafranca, 1954) and the time at which this occurs corresponds with the period during which there is a twofold to threefold increase in the intrinsic speed of shortening of the fast muscles (Close, 1964). These findings suggest that the speed of shortening may be proportional to the rate of hydrolysis of adenosinetriphosphate by myosin.

It is not clear whether the time course of the active state is functionally dependent upon the intrinsic speed of shortening or whether there is some other basis for the observed relation. Whatever the nature of the processes of excitation-contraction it seems likely that following a single stimulus the duration of the active state is determined in one of two general ways. In the first place the contractile material might be supplied with a certain amount of activator (e.g. Bianchi & Shanes, 1959) which is utilized within a certain time depending upon the intrinsic speed of shortening (Close, 1964). Alternatively the contractile material may be allowed to operate for a certain time, the duration of which is governed by some extrinsic process (e.g. Hill & Macpherson, 1954). In this case it is necessary to postulate that the intrinsic speed of shortening and the time

course of the active state are coupled indirectly, either inherently or functionally, in such a way that a hyperbolic relation exists between the two. In any event, the duration of the active state could not determine the speed of shortening. Furthermore, the actual speed of shortening of the whole muscle or sarcomere does not control the duration of contraction. In this connexion it is necessary to distinguish clearly between changes in speed of shortening due to alterations of the load and differences in the intrinsic speed of shortening as defined above. In isotonic twitches, for example, a decrease in speed of shortening which is brought about by an increase in the load is not associated with an increase in the isotonic-twitch contraction time or in the time at which the peak of the twitch occurs after the stimulus.

If the time course of decay of the active state were dependent upon the rate of utilization of an activator, proportional to the intrinsic speed of shortening, the factor k would be proportional to the amount of activator utilized. The role of calcium ions in activation of the contractile elements may be important in this connexion. The uptake of calcium ions has been shown to be proportional to the size of contractions and the amount of work done (Bianchi & Shanes, 1959; Winegrad & Shanes, 1962; Niedergerke, 1963; Langer & Brady, 1963). For this reason it is probable that calcium ions are involved in the process of activation and it is possible that the amount of calcium released into the muscle fibre during a twitch is proportional to k. However, it is not known whether these calcium ions act directly upon the contractile material or at some other site. Alternatively, if the active state is only indirectly related to the intrinsic speed of shortening and its time course of decay is controlled by some extrinsic process, k may be proportional merely to the amount of substrate utilized during the twitch.

The results of the present work show that there is a hyperbolic relation between the intrinsic speed of shortening and the isometric-twitch contraction time but they do not provide any information on the nature of the mechanism underlying this relation. It remains to be demonstrated whether the time course of decay of the active state is dependent upon the intrinsic rate of contraction or whether there is some other basis for the relation. A comparative study should be made of various muscles not only to determine the relation between the intrinsic speed of shortening and the rate of hydrolysis of adenosinetriphosphate by myosin, but also to test the possibility that the uptake of calcium during contraction is independent of changes in the intrinsic speed of shortening.

SUMMARY

1. An attempt has been made to determine the effect which changes in the intrinsic speed of shortening may have upon the time course of decay of the active state.

2. The speed of shortening of new-born rat extensor digitorum longus muscles *in vitro* at different temperatures and the developmental changes in the speed of shortening of mouse and rat muscles were examined.

3. It has been shown that changes in the intrinsic speed of shortening are associated with inversely proportional changes in the isometric-twitch contraction time but do not lead to a change in the twitch: tetanus ratio.

4. Evidence is presented which indicates that the principal difference in the time courses of the isometric twitches of many different muscles may be attributed to differences in their intrinsic speeds of shortening.

5. It is concluded that there is a hyperbolic relation between the intrinsic speed of shortening and the duration of the active state.

6. Some mechanisms are discussed which might underlie this relation.

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