J. Physiol. (1960), **150**, *pp.* 399–416
With 12 *text-figures*
Printed in Great Britain

DIFFERENTIATION OF FAST AND SLOW MUSCLES IN THE CAT HIND LIMB

BY A. J. BULLER, J. C. ECCLES AND ROSAMOND M. ECCLES*
From the Department of Physiology, Australian National University,
Canberra, Australia

(*Received* 1 *September* 1959)

Ranvier (1874) described the first systematic investigation of the contractions of the slow and fast limb muscles, and a further systematic investigation was made by Fischer (1908). Banu (1922), Denny-Brown (1929*b*) and Koschtojanz & Rjabinowskaja (1935) showed that all limb muscles were slow at birth and differentiation into the fast and slow types occurred in mammals during the first few weeks after birth. Recently it has been found that motoneurones innervating slow muscles have as a rule much longer after-hyperpolarizations following the discharge of an impulse than do the motoneurones supplying fast muscles (Eccles, Eccles & Lundberg, 1958). Thus the characteristic frequencies of discharge of these two types of motoneurones (Denny-Brown, 1929*a*; Granit, Henatsch & Steg, 1956; Granit, Phillips, Skoglund & Steg, 1957) are explicable by their intrinsic properties. The shorter after-hyperpolarization of motoneurones supplying fast muscles allows their fast frequency of firing, which is appropriately related to the contraction time of their muscles; and complementarily those motoneurones with longer after-hyperpolarizations have as a consequence frequencies of discharge appropriate to the slow muscles they innervate. It was, therefore, of interest to re-investigate the process of differentiation into fast and slow muscles, in an attempt to determine whether this appropriate matching of motoneurones to muscles was brought about by motoneurones influencing muscle differentiation, or, vice versa, by muscle influencing motoneurones.

The present paper describes a detailed study of muscle differentiation as defined by various measurements of the speed of muscle contraction. It also describes the effects of various nerve lesions on this differentiation. A more analytical investigation is described in the following paper (Buller, Eccles & Eccles, 1960).

---

* Nuffield Travelling Fellow of the Royal Society. Present address: St Thomas's Hospital Medical School, London, S.E. 1.

METHODS

The animals (kittens and cats) were anaesthetized with pentobarbital sodium. The muscles under investigation were isolated by dissection, care being taken to preserve their blood supply. The motor nerves were cut centrally and mounted on stimulating electrodes. Full precautions have been taken to ensure that the muscle contractions approximated as closely as possible to isometric contractions: for the leg muscles steel drills transfixed the distal end of the femur and the distal ends of the tibia and fibula; for the thigh muscles the drills were inserted through the greater trochanter and the distal end of the femur. The drills were clamped in chucks which in turn were fixed to a massive steel frame to which the recording strain gauge (Statham Gl–8–350 or Gl–80–35) was also fixed by an assembly giving great flexibility of adjustment. The muscle tendon (or the patella in the case of crureus) was tied firmly to a short steel hook directly linked to the strain gauge; care was taken to align the strain gauge with the direction of muscle pull, which of course was kept as close as possible to its natural line of pull. The direction of muscle pull was about 60° from the vertical so that it was possible to have the muscle immersed in a paraffin pool with its motor nerve readily accessible. Controlled heating and stirring of the pool ensured that its temperature was almost always in the range 37–38° C. This temperature control was important because the speed of muscle contraction has a fairly high temperature coefficient; $T_{10} = 1{\cdot}53$ according to Gordon & Phillips (1953). Despite the oblique angle of the muscle pull there was a negligible sagging of the muscle, because its weight was greatly reduced by its immersion in paraffin and it had a fairly high initial tension—usually more than ten times the weight of the muscle.

Considerable effort has been made to ensure that the various muscles were contracting under comparable conditions of initial tension. The relationship of initial tension to duration of the isometric twitch was described in detail by Fulton (1925, 1926), but unfortunately the twitches were distorted by friction of the myograph bearing, as revealed by the angle between the summit and the falling phase (Cooper & Eccles, 1929). A re-investigation was therefore necessary, and Fig. 1 shows the effect of length on the initial tension and on the time course and size of the twitch. Measurements from the series partly shown in Fig. 1*A–E* are plotted in Fig. 1*F* with the muscle lengths as abscissae; and as ordinates the initial tensions, the twitch tensions, the twitch contraction times (onset to summit), and the half-decay times (summit to half-decay). It will be seen that around the optimum condition for active tension development (cf. Banus & Zetlin, 1938) progressive lengthening of the muscle causes lengthening both of the contraction time of the twitch and of the time from summit to half-relaxation. It was therefore necessary to adopt some criterion in order that the contractions of different muscles could be compared under standard conditions. The standard adopted was the muscle length giving the maximum twitch tension, as indicated by the vertical line in Fig. 1*F*. In all figures zero on the tension scales has indicated zero muscle tension.

The muscle contractions have been evoked by stimulating the appropriate muscle nerve with brief electrical pulses about 0·1 msec in duration and about twice maximum strength. Several twitches were recorded at each length of the muscle, but measurements were made only of the twitches at the optimum length and all the values plotted are the means of these measurements. A decade-counter device was constructed so that the duration of the twitch contraction time could be read off on the lower beam of the oscilloscope (cf. Figs. 2, 11) at the same time as the upper beam was recording the time course of the twitch (Buller, unpublished). The tetani were recorded only at the initial tension giving optimum twitches, and were evoked by brief repetitive electrical pulses, as for the twitches. Usually the series was from the slowest to the fastest with a final return to the slowest frequency; the frequencies were standardized at 13, 19, 32, 46, 66, 104 a second. There was an interval of several seconds between successive tetani, but not long enough to allow for dissipation of the post-tetanic potentiation of the muscle twitches (Brown & von Euler, 1938; Feng, Lee, Meng & Wang, 1938; von Euler & Swank, 1940). Post-tetanic potentiation was, however, much smaller for tetani, so no large error was introduced by calculating the tetanus-to-twitch ratios relative to the twitch height at the onset of the tetanic investigations. The durations of the tetani were kept very short (about 0·2 sec) so as to minimize post-tetanic potentiation.

In separating lateral from medial gastrocnemius it is necessary to divide a small slip of muscle that arises from a common tendon. Separation between lateral gastrocnemius and plantaris is not possible by dissection because both muscles have a large origin from a common tendon. No appreciable error has been introduced thereby, because these muscles normally respond with twitches having about the same time courses and they have not been employed in the more analytic procedures involving nerve cross-unions, as described in the subsequent paper (Buller *et al.* 1960). The crureus muscle can be separated from rectus femoris and vastus lateralis, but separation from vastus medialis is not possible. Contractions

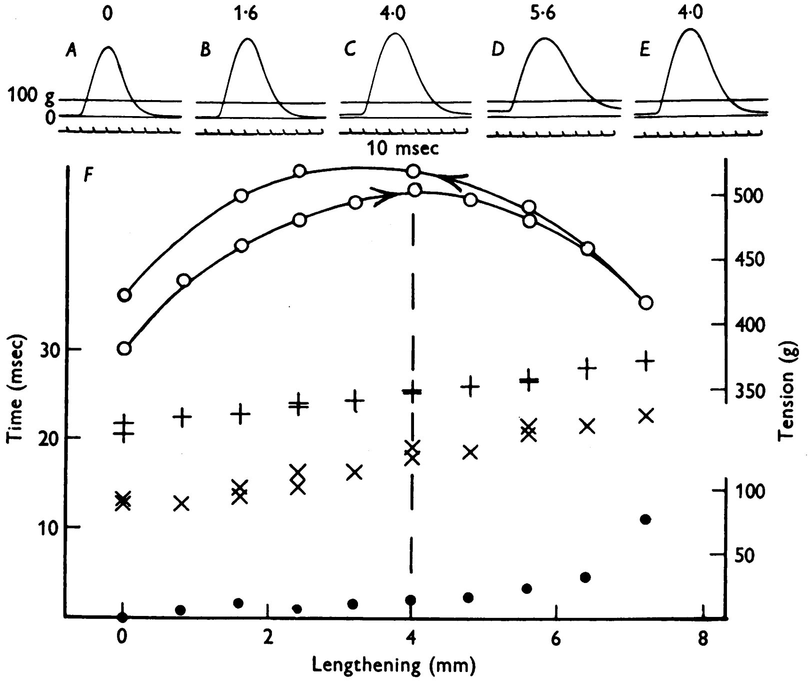

Fig. 1. Isometric twitch contractions of flexor digitorum longus muscle (FDL) of a spinal kitten 13 weeks old. Progressive lengthening from an arbitrary zero length in *A*, the successive lengthenings being 1·6 mm for *B*, 4·0 mm for *C* and 5·6 mm for *D*, with a return in *E* to the same length as *C*. Note tension scale of 100 g. Weight of kitten = 1·5 kg. In *F* the whole series partly shown in Fig. 1*A–E* is plotted with the lengthenings as abscissae; and as ordinates, the initial tension (●), the twitch tensions (○), the contraction times, i.e. times from onset to summit of the twitch (+) and the half-relaxation times, i.e. times from summit to half-relaxation (×). Throughout this paper the various measurements on the muscle twitches have been made under the conditions giving the greatest twitch contraction, i.e. at the vertical line in Fig. 1*F*. Twitch tensions were rather higher on the return from the greatest lengthening, but muscles were not ordinarily subjected to such excessive stresses. Note break in tension scales (right-hand ordinate).

of crureus independently of vastus medialis were secured by cutting all nerve branches to vastus medialis. As a check on the effectiveness of this separation of the slow muscle, crureus, from the fast muscle, vastus medialis, the whole of the branch from quadriceps nerve to crureus and vastus medialis was often cut after the contractions of crureus had been recorded, leaving intact only the crureus nerve that arises from the nerve to vastus lateralis. Invariably it was found that this fraction of crureus gave twitches and tetani closely comparable to those of the whole crureus. All other muscles were readily isolated.

RESULTS

Normal differentiation of fast and slow muscles

Muscle twitches in a representative series of muscles are shown in Fig. 2 for animals ranging from 20 hr after birth to 28 weeks. In confirmation of Banu (1922) and Denny-Brown (1929*b*), all muscles are equally slow in the new-born kitten (*A*), with a contraction time of about 80 msec and a half-relaxation time also of about 80 msec. Within a few days the fast muscles are clearly differentiated from the slow muscles, soleus and crureus (Fig. 2*B*), and by about four weeks (Fig. 2*C*) the fast muscles have attained a speed which is virtually unaltered thereafter, as is indicated by Fig. 2*D* at

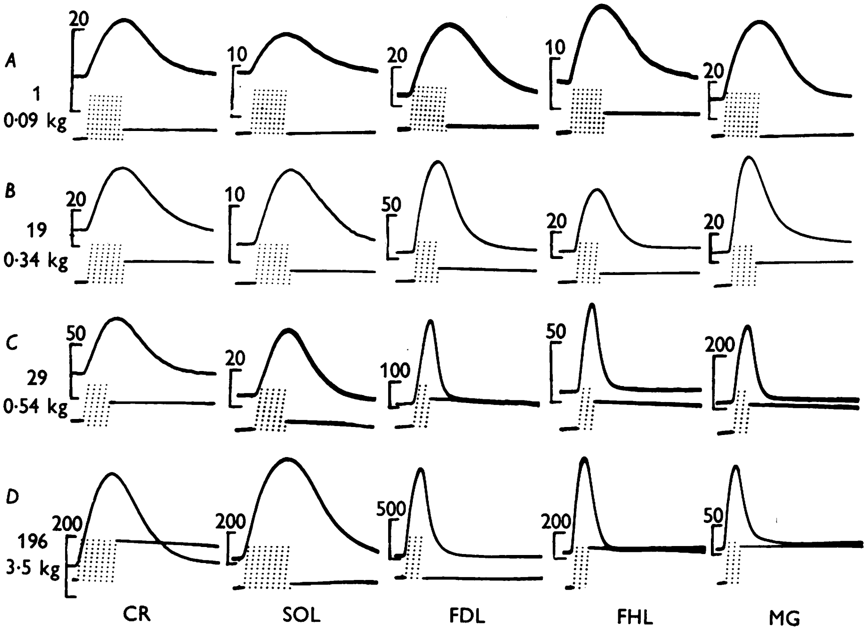

Fig. 2. *A*–*D*. Isometric twitch contractions of five different leg muscles in the cat at the ages in days and weights shown, and with contraction times indicated in milliseconds by the decade-counter device. CR = crureus; SOL = soleus; FDL = flexor digitorum longus; FHL = flexor hallucis longus; MG = medial gastrocnemius. Tension scales indicated in grams, zero giving zero tension on muscle.

28 weeks. It appears from Fig. 2*C* that in the 4-week kitten the slow muscles are appreciably faster than in the 28-week kitten, and this effect is well displayed in the plotted series of Figs. 3*D* and 4*D*, both for contraction times and half-relaxation times. The points for the two slow muscles are seen to lie about curves of shortening contraction and relaxation times until minima of just over 50 msec for both are attained at about 5 weeks, but thereafter the twitches become slower and after several weeks reach the adult values of about 70 and 75 msec respectively. On the whole the crureus tends to be a little faster than soleus (cf. Gordon & Phillips, 1953). In Figs. 3*A*–*C* and 4*A*–*C* the six fast muscles are seen to have comparable time courses of differentiation, and the curves drawn in Figs. 3*A*–*C* and 4*A*–*C* are in fact the same mean curves derived respectively from the pooled result of the six muscles. The standard curve of fast-muscle differentiation reaches a minimum value of about 25 msec contraction time and 16 msec half-relaxation time at 8–16 weeks, but thereafter there is probably a slight lengthening to the adult values of about 27 and 18 msec respectively.

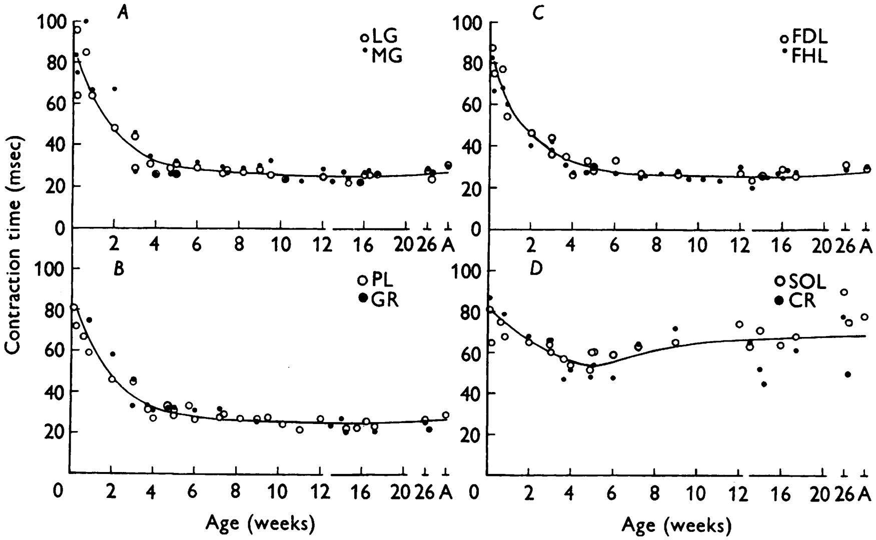

Fig. 3. Contraction times of the various muscles as ordinates against ages of cats as abscissae for six fast muscles, as indicated by the symbols in *A*, *B*, *C* and for two slow muscles in *D*. Muscles additional to those of Fig. 2 are lateral gastrocnemius, LG, plantaris, PL, and gracilis, GR in *B*. The curves in *A*, *B* and *C* are the same curve that is drawn for the pooled results of these three graphs for the six fast muscles. Note change in age scale beyond 12 weeks, and the final *A* values for adult cats. Usually each of the plotted points represents the mean for the muscles on the two sides. The points at 26 weeks, 26+ weeks, and adults represent the means for several animals, usually three.

In Fig. 5*C*, *D*, *E* it is seen that already in the 3-week-old kitten the responses of the fast muscles to repetitive stimulation have approximated to the adult pattern. There is relatively little summation at 13/sec, and with a frequency as high as 46/sec the rhythm of stimulation can be detected on the tetanic plateau. In contrast, the rhythm of stimulation has virtually disappeared at 32/sec with the two slow muscles, crureus and soleus (*A*, *B*). When comparing tetani at various frequencies of fast and slow muscles, it will be seen that comparable degrees of fusion occur when the frequencies are appropriately chosen. For example the fusion for fast muscles at 32/sec in Fig. 5*C*–*E* is approximately the same as at 13/sec for the slow muscles (*A*, *B*), and again 46 and 66/sec for the fast muscles correspond respectively to 19 and 32/sec for the slow muscles. However, the comparison is not exact because slow muscles have a slowly climbing tetanic plateau, whereas with fast muscles the plateau shows little or no tendency to climb (Fig. 5*C*–*E* at 66/sec), and may even decline.

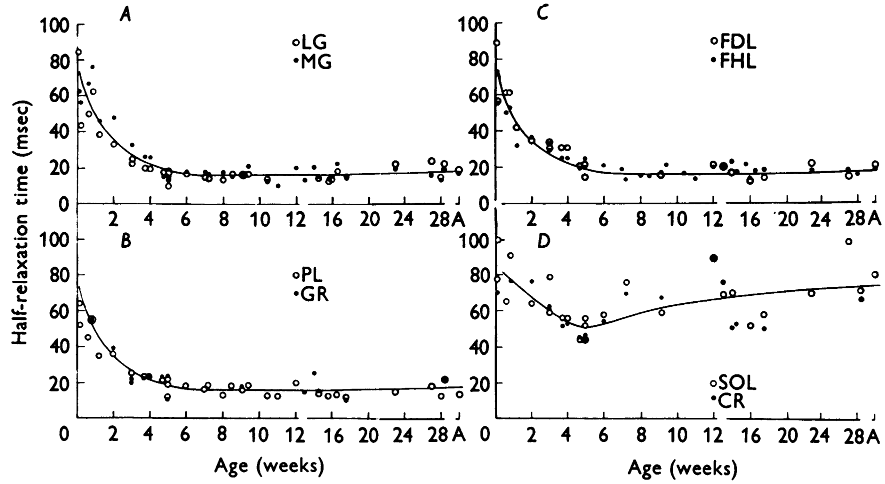

Fig. 4. Half-relaxation times of the various muscles, conventions as for Fig. 3. Note again that the line drawn in *A*, *B* and *C* is for the pooled results of these three graphs for the six fast muscles. Again the age scale is changed beyond 12 weeks.

When comparing the speeds of different muscles, as exhibited by their responses to various frequencies of stimulation, it has been important to plot the response–frequency curves for these muscles, or, response–stimulus-interval curves as has been done in Fig. 5*F*–*J*. The frequencies or stimulus intervals are related to the tensions of the summed contractions by S-shaped curves (best seen in *I*, *J*) that attain maxima at frequencies which give a smooth plateau, i.e. at which there is complete fusion of the summed contraction responses (cf. Adrian & Bronk, 1929; Cooper & Eccles, 1930). A basis for comparison of the speeds of muscles has been conveniently given by the stimulus intervals for the mid points of the S-shaped curves, as shown by the arrows in Fig. 5*F*–*J*. The characteristic stimulus intervals so determined for the various muscles have been plotted in Fig. 6 against age, so forming another set of curves expressing the time courses of differentiation of muscles (cf. Figs. 3, 4). In the day-old kitten the repetitive responses reveal that all muscles are uniformly slow (cf. Fig. 7), and the adult speed of fast muscles is almost attained by five weeks. The curves of Fig. 6 are less complete than Figs. 3 and 4 because the strain gauge was too weak (maximum only 2·2 kg) to record the tetanic contractions of most fast muscles in kittens of over 16 weeks. A further defect in Fig. 6 is that the durations of the tetani were usually too brief to attain the plateau tensions with the slow muscles, particularly at low frequencies (cf. Fig. 7). As a consequence the plotted points of Fig. 6*D* are often too low, and this may account for the very low values for soleus and crureus at 1–2 weeks.

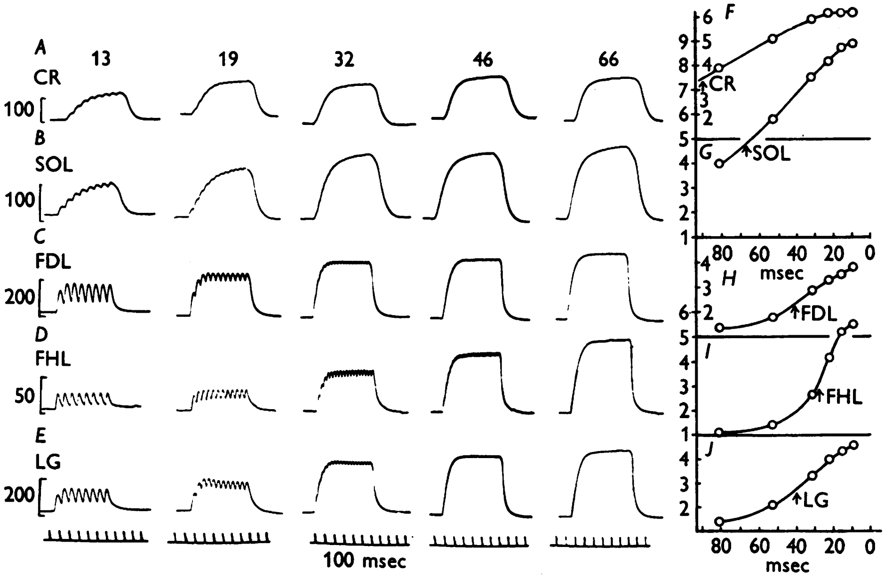

Fig. 5. *A*–*E*. Responses of five representative muscles of a 3-week kitten, as indicated, to repetitive stimulation at the frequencies per second stated above each column. Note tension scales in grams. At the column on the extreme right (*F*–*J*) the tetanus: twitch ratios for each muscle are plotted as ordinates against the stimulus intervals of the tetani (i.e. the reciprocals of the frequencies), as abscissae. Note that the longest stimulus intervals are plotted to the left to correspond with the arrangement of the records in each row. The arrows mark on the response–stimulus-interval curves of each muscle the stimulus intervals giving half the maximum tetanic summation, i.e. half way between the twitch tension and the maximum tetanic tension. Weight of kitten, 0·52 kg.

Despite these defects, Fig. 6 may be regarded as giving a sufficiently reliable index of the differentiation of fast and slow muscles in respect of their repetitive responses, which is seen to follow a time scale approximately comparable with that given by the two previous criteria, contraction times (Fig. 3), and half-relaxation times (Fig. 4).

The unique time courses of differentiation of the speeds of contraction of fast and slow muscles raise the question of the nature of the controlling influences. For example, it can be asked if the influences are inherent in the muscles or if they are the result of some action of the nervous system which is different for fast and slow muscles. As a first attempt to decide between these two alternatives, the nervous system has been subjected to various operative procedures in kittens a few days old and the effects of such lesions on muscle differentiation have been examined by later experimental investigations. One kitten could of course be employed only for one such testing period, just as with the normal differentiation curves of Figs. 3, 4, 6.

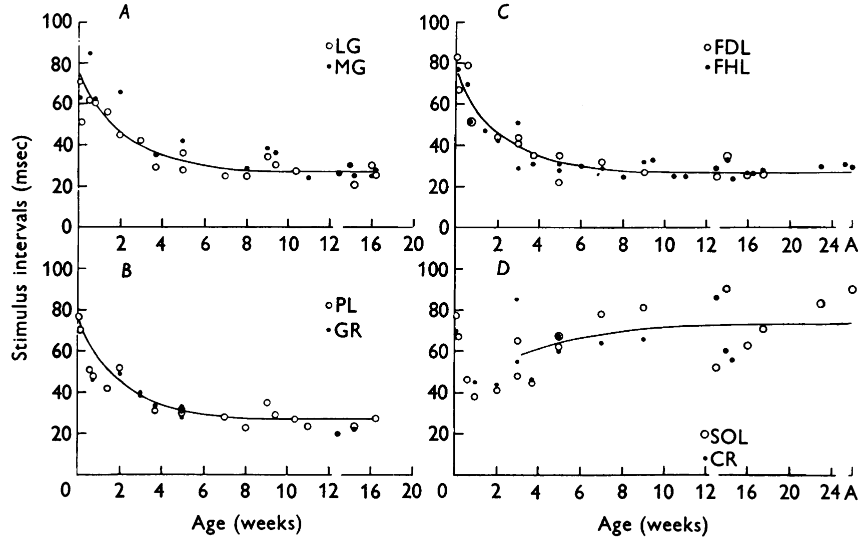

Fig. 6. Stimulus intervals, giving half the maximum tetanic summation, measured as indicated in Fig. 5*F–J*; conventions as for Fig. 3. There is a paucity of values for the older animals because the maximum tetanic tensions were beyond the range of the strain gauge.

Effect of spinal cord transection on differentiation of muscle

The spinal cord was transected at the first or second lumbar segment in nine kittens 1–3 days old. A representative series of muscle twitches is illustrated in Fig. 8 for a spinal kitten 13 weeks old. With normal kittens

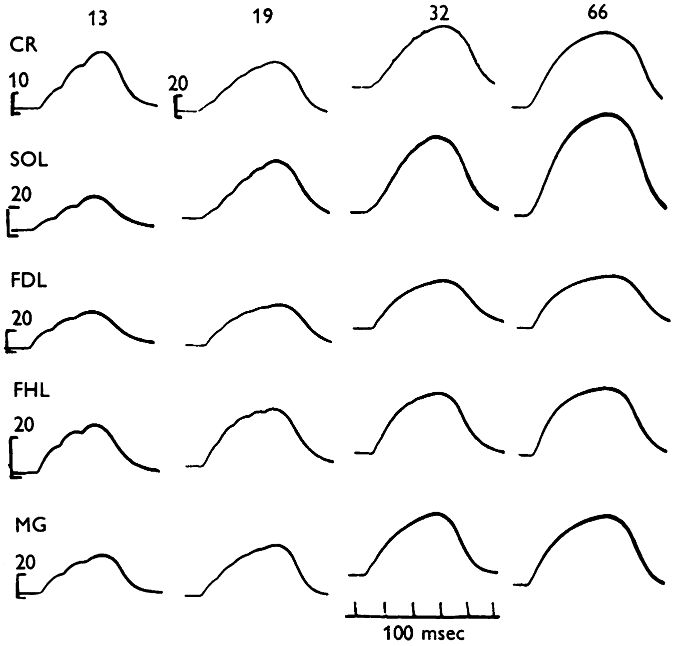

Fig. 7. Tetanic responses of the various muscles of a kitten 20 hr old at the frequencies indicated as in Fig. 5; note that the crureus muscle at the lowest frequency was recorded at higher amplification than the remainder of the series. Tension scales in grams; weight of kitten, 0·09 kg.

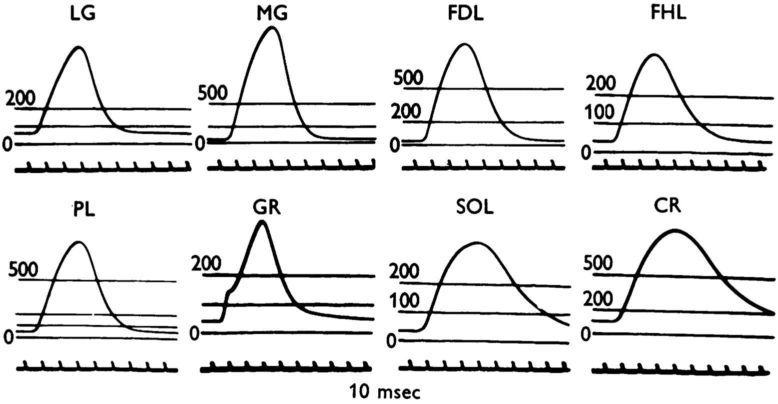

Fig. 8. Twitches of the various muscles indicated in a 13-week-old kitten, spinal cord transected at the L1 level when 36 hr old; note the very fast twitches for crureus and soleus muscles, and the very fast relaxations of the gastrocnemii, and gracilis; the two components of gracilis are very conspicuous. Weight of kitten, 1·75 kg; tension scales in grams, showing as usual the zero tension.

of that age there has been virtually complete differentiation of the slow muscles, the contraction times being in the range 60–90 msec (cf. Fig. 3*D*), and the half-relaxation times 50–100 msec (cf. Fig. 4*D*). In contrast, crureus and soleus in Fig. 8 had contraction times of 33 and 32 msec respectively, and half-relaxation times of 24 and 25 msec respectively. The observations on soleus and crureus contractions in spinal kittens are plotted as open and filled circles in Fig. 9*D* for contraction times, and in Fig. 10*D* for half-relaxation times. Comparison with the standard curves of Figs. 3, 4 (broken lines) reveals that there has been a failure of the differentiation of slow muscles that normally occurs between 5 and 16 weeks; as a consequence soleus and crureus responded by twitches that were but little slower than the fast muscle responses (cf. Fig. 8). Correspondingly there was a shortening of the stimulus interval for half tetanic summation, the mean value at 10–20 weeks being about 40 msec, which is much closer to the mean value for normal fast muscles, 27 msec, than for normal slow muscles, 73 msec (Fig. 6).

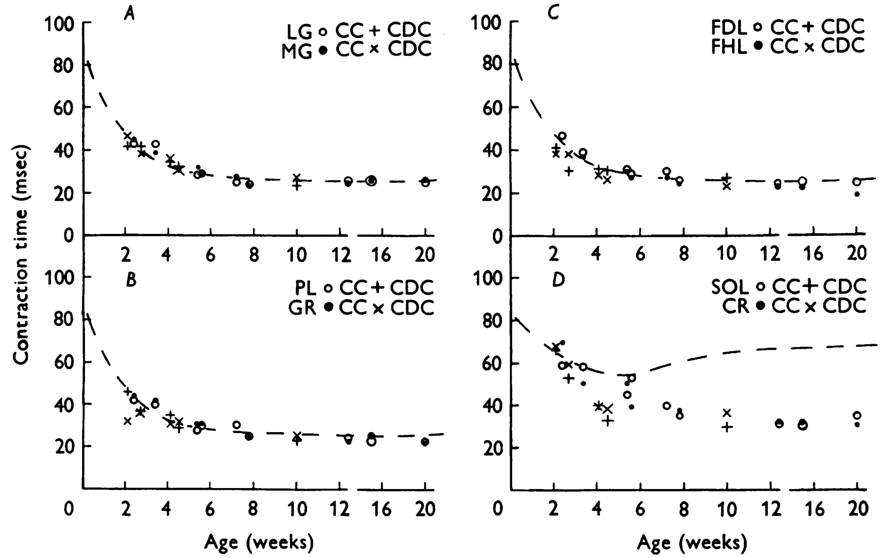

Fig. 9. The open and filled circles show the contraction times for muscle twitches, as in Fig. 3, but for animals in which the spinal cord had been transected when 1–4 days old, as illustrated in Fig. 8 (CC, cord cut; CDC, cord and dorsal roots cut). The crosses show a similar series for muscles as indicated by the symbols, but for animals in which the initial operation gave a complete isolation of the spinal cord from all incoming impulses, as described in the text, a representative series being illustrated in Fig. 11 (CDC = cord and dorsal roots cut). The broken lines show the standard curves for the contraction-time–age curve, derived as in Fig. 3.

On the other hand the contraction times of the fast muscles of spinal kittens differed very little from the muscles of normal kittens (Fig. 9*A*, *B*, *C*), though possibly the contraction times were a little shortened in the oldest spinal kittens. A much more significant difference appeared in the half-relaxation times of these muscles, particularly the medial and lateral gastrocnemii (Fig. 8), plantaris and gracilis (Fig. 8), the mean values being about 12 msec (Fig. 10*A*, *B*), as against 16–18 msec for normal kittens in the age group of 6–20 weeks (Fig. 4*A*, *B*). As is illustrated in Fig. 8 and plotted in Fig. 10*C*, the half-relaxation times for the FDL and FHL muscles were unaffected by the spinal transection, with mean values of about 17 msec over the same range of age. The early step on the gracilis twitch appears when there is a slack component (R. M. Eccles & Iggo; unpublished).

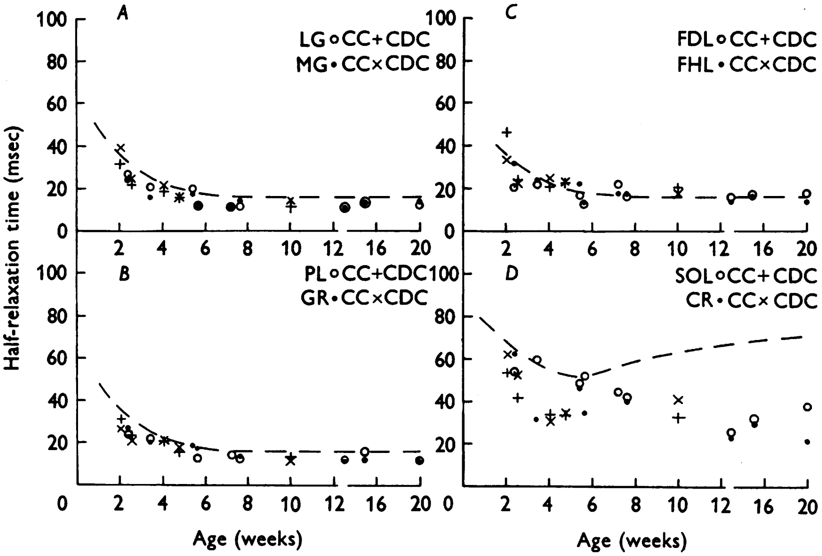

Fig. 10. Half-relaxation times of the muscle twitches; conventions as in Fig. 9. The broken lines show the standard curves as derived in Fig. 4.

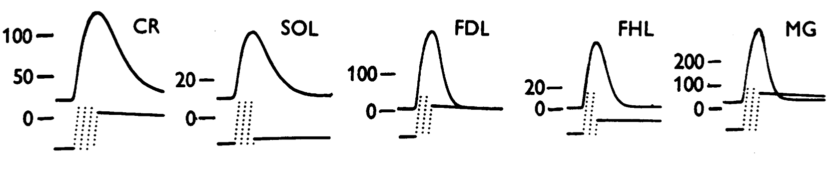

Fig. 11. Twitches for the various muscles in a 33-day-old kitten in which the spinal cord was subjected to an 'isolation' operation, as described in the text, when 4 days old; note the very fast twitches given by crureus and soleus muscles. Weight of kitten, 0·51 kg; tension scales in grams.

The stimulus intervals for half tetanic summation of fast muscles also were not significantly affected by the spinal transection.

Severance of all inflow of impulses into lower lumbar spinal cord

A more complete isolation of the lumbo-sacral cord was accomplished in five kittens by an initial operative procedure of eliminating all afferent input into the spinal cord by severing the dorsal roots extradurally on both sides of the stump of cord below the transection in the upper lumbar region, the ventral roots being left intact. This operation has previously been employed in order to effect a complete suppression of the discharge from all motoneurones, so producing disuse atrophy of the muscles (Tower, 1937*a*, *b*, 1939; Eccles, 1941, 1944). Kittens less than 1 week old adapted to this severe operation much better than older kittens. These completely inactivated muscles showed a remarkable gain in weight and power, so that over the first 5 weeks they did not fall below two-thirds of normal in these respects.

The final experimental tests (cf. Fig. 11) revealed that the differentiation of fast muscles proceeded along virtually the normal time course (crosses in Figs. 9*A–C*, 10*A–C*), though, just as with the transected spinal cord, there was possibly a little deviation towards shortening at the longest post-operative intervals. However, with soleus and crureus the contraction times, half-relaxation times and the stimulus intervals for half tetanic summation showed an even more rapid shortening over the first few weeks (cf. Fig. 11 at 33 days old) than was observed after the spinal transection (cf. crosses with circles in Figs. 9*D*, 10*D*). As a consequence, the time courses of differentiation of these muscles (Figs. 9*D*, 10*D*) differed very little from those of fast muscles (Figs. 9*A*, *B*, *C*; 10*A*, *B*, *C*).

Cutting of dorsal roots

Since complete isolation of the spinal cord from incoming impulses produced a larger change in the slow-muscle differentiation than mere section of the spinal cord, it was of interest to see if muscle de-afferentation *per se* would cause any change in muscle differentiation. The dorsal roots of the lumbosacral spinal cord were transected extradurally from L4 to S2 on one side, the other side being left as a control.

Two kittens were tested at 12 weeks after such an operation had been performed 2–3 days after birth. The contraction times were found to be virtually identical on the two sides for the fast muscles, as also were the half-relaxation times and the stimulus intervals for half tetanic tension. In contrast, for the de-afferented slow muscles, soleus and crureus, the mean values for all these three times were 10 to 15 % lower than the control values. More investigation is desirable, but it seems likely that de-afferentation of slow muscles causes a small effect of the same kind as that observed with cord section or isolation of the spinal cord.

DISCUSSION

In kittens a few days old the operative isolation of the lumbosacral spinal cord from all incoming impulses has resulted in completely flaccid hind-limbs. As with adult animals (Tower, 1937*a*, *b*, 1939; Eccles, 1941), there has apparently been an absence of all discharge of impulses from the motoneurones. Under such circumstances it was remarkable that the muscles developed so well in size and in power of contraction. Furthermore, the development of speed of contraction of fast muscles followed a normal time course over the first few weeks (crosses in Figs. 9*A*, *B*, *C*; 10*A*, *B*, *C*); thereafter the contractions were rather faster than normal, particularly during the relaxation phase (Figs. 10*A*, *B*). It can be concluded that the normal process of differentiation of fast muscles occurs independently of their activity. As would be expected, the differentiation of fast muscles was also unaffected by partial isolation of the lumbosacral spinal cord from incoming impulses, either by transection of the spinal cord (circles in Figs. 9*A*, *B*, *C*; 10*A*, *B*, *C*), or by de-afferenting it on the side of the muscles being investigated.

It is of great interest that the slow muscles, soleus and crureus, exhibited such a different behaviour. There was a complete failure of the late phase of slowing of contraction and relaxation (Figs. 9*D*, 10*D*), which normally resulted in the full differentiation of the slow muscles during the age range of 5–16 weeks (Figs. 3*D*, 4*D*). Nevertheless, soleus and crureus remained throughout a little slower than the fast muscles, particularly in their relaxation phase. It seems likely, therefore, that they were subjected to a small differentiating influence even after the operation of complete isolation of the spinal cord. Over the age range of 3–8 weeks the contraction times and half-relaxation times for soleus and crureus tended to be briefer after the operation of complete isolation of the spinal cord than when there was merely a cord transection (compare crosses with circles in Figs. 9*D*, 10*D*). Possibly the spinal transection did not accomplish a full removal of all of the differentiating influence that is normally exerted on soleus and crureus. It seems that de-afferentation is still less effective in removing this differentiating influence, but further investigation is desirable.

The operative transection or isolation of the spinal cord in addition depressed the summation of contractions both of fast and slow muscles. This effect has been observed to develop within 3 weeks of the isolated cord operation in adult cats, the tetanus: twitch ratio being usually reduced to about half the normal value (Eccles, 1941). A similar reduction of the tetanus: twitch ratio was observed when the spinal cord isolation or transection was performed on 5-week-old kittens (Buller *et al.* 1960). In the adult cat daily stimulations of the muscle caused little or no improvement in the tetanus:twitch ratio, in contrast to the considerable improvement in disuse atrophy that was produced, particularly in flexor muscles (Eccles, 1941). It does not, therefore, seem justifiable to attribute the low tetanus:twitch ratios simply to disuse of the muscles. Yet it is to be distinguished from the effects of the spinal cord operations on the speeds of muscles, for it occurs with fast as well as with slow muscles.

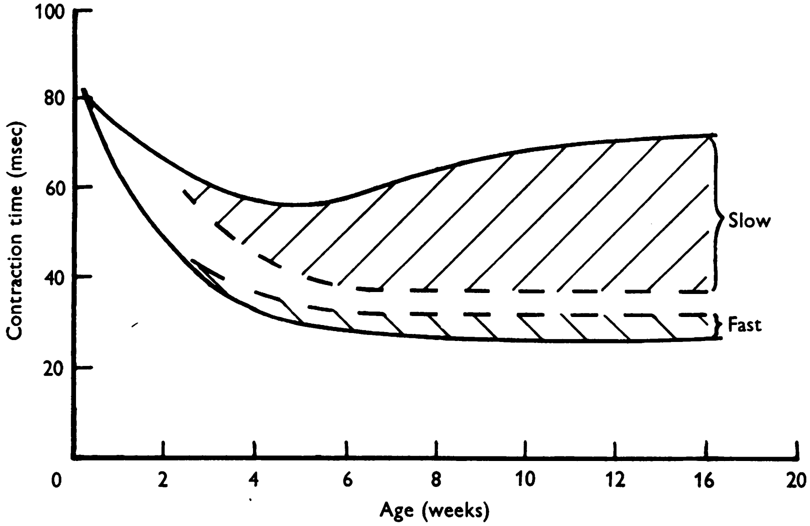

Fig. 12. Diagram showing the standard time courses of the contraction-time–age curves (Fig. 3) for slow and fast muscles (continuous lines), and the postulated curves for these muscles in the absence of neural influences (broken lines). The hatched areas thus give the effects of the respective neural influences.

Under all experimental conditions the contractions of all muscles except gracilis have indicated that there has been no appreciable admixture of muscle fibres with very different contraction and relaxation times. Evidence of an admixture of fast and slow muscle fibres has been obtained for tibialis anticus (Gordon & Holbourn, 1949; Gordon & Phillips, 1953) and for caput medialis of triceps brachii (Eccles *et al.* 1958). It might appear that with gracilis there is an extremely fast component with a contraction time of about 5 msec (Fig. 8 GR). However, recent investigation (R. M. Eccles & Iggo, unpublished) has shown that this effect arises on account of the relative slackness of a component of gracilis that is inserted into the fascia over the gastrocnemius.

It will be convenient to introduce at this stage a diagram (Fig. 12) illustrating the postulated action of differentiating influences on slow and fast muscles, though some of the supporting evidence is given in the next paper (Buller *et al.* 1959). The two continuous lines are the standard curves for the differentiation of slow and fast muscle contraction times (Fig. 3). The broken lines give the postulated curves when the slow and fast differentiating influences have been eliminated by the operation of the isolated spinal cord. The broken line for slow muscles is that observed in Fig. 9*D*, and the effect of the differentiating influence is portrayed by the hatched area. With fast muscles the isolated cord operation failed to reveal any comparable influence (Fig. 9*A–C*), but when the isolated cord operation was performed on 5-week-old kittens, the fast muscles had contraction times about 5 msec slower than normal (Buller *et al.* 1960, Fig. 11*A*). Possibly the action of disuse during the first few weeks of differentiation had prevented the appearance of these slightly slowed contraction times in Fig. 9. Certainly the small hatched area representing a fast differentiating influence is based upon much less secure evidence than is the slow differentiating influence. Figure 12 also depicts, by the difference between the two broken lines, the small factor of slowness or fastness that remains to characterize the slow and fast muscles after isolation or transection of the spinal cord (Figs. 9, 10); and which is also evidenced by the cross-union experiments of the next paper (Buller *et al.* 1960).

In one respect Fig. 12 may be related to earlier experimental observations. The suggested absence of a differentiating influence from the spinal cord with kittens under 2 weeks old conforms with the general finding of experimental embryology that nerves do not exert a formative influence until late in embryonic life (Hamburger & Levi-Montalcini, 1950; Singer, 1952). The same independence of peripheral tissues has been demonstrated by the good development of muscles, both in weight and contraction tension, that have been completely inactivated from a few days after birth.

The experimental investigations of Gasser & Hill (1924) and of Hill (1949, 1953) on the frog sartorius have indicated that the 'active state' of the muscle contractile process is no greater for a fused tetanus than for a twitch. The recorded 'isometric' contractile tension of the twitch is so much lower because the series-elastic components of the muscle allow shortening even under very rigid extrinsic conditions of isometricity (Hill, 1949; Jewell & Wilkie, 1958); and, as a consequence of the shortening and the muscle viscosity, the rising phase of the twitch contraction is delayed and reaches its maximum only when the 'active state' has subsided to a fraction of its maximum (Hill, 1949) that depends on the particular muscle being studied. On the other hand, during a tetanus at a sufficiently high frequency, the 'active state' is maintained at a steady level, which gives the tetanic tension during a maintained plateau. No attempt has yet been made to determine the time courses of the 'active states' during twitches of mammalian fast and slow muscles. However, since these muscles exhibit similar differences in time course, not only in contraction time (Figs. 3, 9), but also in half-relaxation time (Figs. 4, 10) and in the stimulus intervals for a corresponding summation of tetanic responses (Fig. 6), it may be assumed that the time courses of their 'active states' are involved in the differences in their speeds of contraction. From the difference in the rate of rise of tension in a tetanus, it appears that the force–velocity relation may also be implicated. Thus the normal differentiation into slow and fast muscles and the changes produced in this by the operative procedures on the spinal cord arise on account of changes in the durations of the 'active states' and in the contractile process of the muscles. The techniques developed by Macpherson (1953), by Macpherson & Wilkie (1954), and by Ritchie (1954) should be employed in order to analyse these differences for the fast and slow muscles under the various conditions of investigation.

SUMMARY

1. Isometric twitch and tetanic responses of hind-limb muscles of cats have been recorded over a wide range of ages, from within one day of birth to the adult. Precautions have been taken to ensure that the responses have been compared under standard conditions of temperature (37–38° C) and initial tension.

2. Earlier observations have been confirmed in that all muscles are equally slow in the new-born animal, and the fast muscles attain their adult speed in about 6 weeks. The slow muscles, soleus and crureus, also quicken, but to a lesser extent, over the first 5 weeks. Thereafter there is a progressive slowing to approach the adult condition in 16–20 weeks.

3. Virtually the same time course for this differentiation of speed is given by the three measurements that have been employed: time to summit of twitch; time from summit to half-relaxation of twitch; interval between stimuli at the tetanic frequency building up to half the maximum tetanic contraction.

4. The differentiation of fast muscles is virtually unaffected by spinal cord transection or by operative isolation of the lumbosacral spinal cord from all incoming impulses. However, the differentiation of slow muscles is greatly depressed, there being a complete failure of the late phase of slowing, so that in a few weeks soleus and crureus become nearly as fast in every respect as normal fast muscles.

5. These results indicate that the differentiation of slow muscles is largely effected by neural influences exerted from the spinal cord.

REFERENCES

ADRIAN, E. D. & BRONK, D. W. (1929). The discharge of impulses in motor nerve fibres. Part II. The frequency of discharge in reflex and voluntary contractions. *J. Physiol.* **67**, 119–151.

BANU, G. (1922). *Recherches Physiologiques sur le Développement Neuromusculaire.* Paris.

BANUS, M. G. & ZETLIN, A. M. (1938). The relation of isometric tension to length in skeletal muscle. *J. cell. comp. Physiol.* **12**, 403–420.

BROWN, G. L. & VON EULER, U. S. (1938). The after effects of a tetanus on mammalian muscle. *J. Physiol.* **93**, 39–60.

BULLER, A. J., ECCLES, J. C. & ECCLES, R. M. (1960). Interactions between motoneurones and muscles in respect of the characteristic speeds of their responses. *J. Physiol.* **150**, 417–439.

COOPER, S. & ECCLES, J. C. (1929). Isometric muscle twitch. *J. Physiol.* **69**, iii–iv.

COOPER, S. & ECCLES, J. C. (1930). The isometric responses of mammalian muscles. *J. Physiol.* **69**, 377–385.

DENNY-BROWN, D. (1929*a*). On the nature of postural reflexes. *Proc. Roy. Soc.* B, **104**, 253–301.

DENNY-BROWN, D. (1929*b*). The histological features of striped muscle in relation to its functional activity. *Proc. Roy. Soc.* B, **104**, 371–411.

ECCLES, J. C. (1941). Disuse atrophy of skeletal muscle. *Med. J. Aust.* **2**, 160–164.

ECCLES, J. C. (1944). Investigations on muscle atrophies arising from disuse and tenotomy. *J. Physiol.* **103**, 253–266.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1958). The action potentials of the alpha motoneurones supplying fast and slow muscles. *J. Physiol.* **142**, 275–291.

FENG, T. P., LEE, L. Y., MENG, C. W. & WANG, S. C. (1938). Studies on the neuromuscular junction. IX. The after effects of tetanization on neuromuscular transmission in cat. *Chin. J. Physiol.* **13**, 78–108.

FISCHER, H. (1908). Zur Physiologie der quergestreiften Muskeln der Säugetiere. *Pflüg. Arch. ges. Physiol.* **125**, 541–583.

FULTON, J. F. (1925). The relation between the durations of the isometric twitch and of the after-action of tetanus. *Proc. Roy. Soc.* B, **97**, 431–443.

FULTON, J. F. (1926). *Muscular Contraction and the Reflex Control of Movement.* Baltimore: Williams & Wilkins.

GASSER, H. S. & HILL, A. V. (1924). The dynamics of muscular contraction. *Proc. Roy. Soc.* B, **96**, 398–437.

GORDON, G. & HOLBOURN, A. H. S. (1949). The mechanical activity of single motor units in reflex contractions of skeletal muscle. *J. Physiol.* **110**, 26–35.

GORDON, G. & PHILLIPS, C. G. (1953). Slow and rapid components in a flexor muscle. *Quart. J. exp. Physiol.* **38**, 35–45.

GRANIT, R., HENATSCH, H. D. & STEG, G. (1956). Tonic and phasic ventral horn cells differentiated by post-tetanic potentiation in cat extensors. *Acta physiol. scand.* **37**, 114–126.

GRANIT, R., PHILLIPS, C. G., SKOGLUND, S. & STEG, G. (1957). Differentiation of tonic from phasic alpha ventral horn cells by stretch, pinna and crossed extensor reflexes. *J. Neurophysiol.* **20**, 470–481.

HAMBURGER, V. & LEVI-MONTALCINI, R. (1950). Some aspects of neuroembryology. In *Genetic Neurology*, WEISS, P. University of Chicago Press.

HILL, A. V. (1949). The abrupt transition from rest to activity in muscle. *Proc. Roy. Soc.* B, **136**, 399–420.

HILL, A. V. (1953). The mechanics of active muscle. *Proc. Roy. Soc.* B, **141**, 104–117.

JEWELL, B. R. & WILKIE, D. R. (1958). An analysis of the mechanical components of frog's striated muscle. *J. Physiol.* **143**, 515–540.

KOSCHTOJANZ, C. & RJABINOWSKAJA, A. (1935). Beitrag zur Physiologie des Skeletmuskels der Säugetiere auf verschiedenen Stadien ihrer individuellen Entwicklung. *Pflüg. Arch. ges. Physiol.* **235**, 416–421.

MACPHERSON, L. (1953). A method of determining the force–velocity relation of muscle from isometric contractions. *J. Physiol.* **122**, 172–177.

MACPHERSON, L. & WILKIE, D. R. (1954). The duration of the active state in a muscle twitch. *J. Physiol.* **124**, 292–299.

RANVIER, L. (1874). De quelques faits relatifs à l'histologie et à la physiologie des muscles striés. *Arch. Physiol. norm. path.* **6**, 1–15.

RITCHIE, J. M. (1954). The duration of the plateau of full activity in frog muscle. *J. Physiol.* **124**, 605–612.

SINGER, M. (1952). The influence of the nerve in regeneration of the amphibian extremity. *Quart. Rev. Biol.* **27**, 169–200.

TOWER, S. S. (1937*a*). Function and structure in the chronically isolated lumbo-sacral spinal cord of the dog. *J. comp. Neurol.* **67**, 109–131.

TOWER, S. S. (1937*b*). Trophic control of non-nervous tissues by the nervous system: a study of muscle and bone innervated from an isolated and quiescent region of spinal cord. *J. comp. Neurol.* **67**, 241–267.

TOWER, S. S. (1939). The reaction of muscle to denervation. *Physiol. Rev.* **19**, 1–48.

VON EULER, U. S. & SWANK, R. L. (1940). Tension changes during tetanus in mammalian and avian muscle. *Acta physiol. scand.* **1**, 203–219.