Effect of Cross-Reinnervation on Physiological Parameters and on Properties of Myosin and Sarcoplasmic Reticulum of Fast and Slow Muscles of the Rabbit

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ABSTRACT Cross-reinnervation of fast (extensor digitorum longus) and slow (soleus) twitch muscles of the rabbit showed essentially complete fast to slow and slow to fast conversion, respectively, 11--12 mo after surgery with respect to a number of physiological parameters including intrinsic shortening, velocity, and isometric twitch time to peak. There was pronounced but incomplete biochemical conversion as judged by Ca$^{2+}$ uptake by sarcoplasmic reticulum, myosin ATPase, alkali lability, and light chain complement. The question of trophic substances of neural origin is discussed in light of the fact that chronic stimulation for 15 wk of a fast muscle produces complete biochemical and physiological conversion to the slow type.

INTRODUCTION

It has been demonstrated that cross union of the nerves to slow (soleus) and fast (extensor digitorum longus) twitch muscles in rats and cats produces reciprocal changes in dynamic properties (Close, 1969; Luff, 1975) and myosin ATPase activity (Bárány and Close, 1971; Buller et al., 1969). The first indication that the light chain complement of cross-innervated (cat) muscle changes came from Samaha et al. (1970). The evaluation of these results is somewhat difficult since no physiological data are available, the myosin ATPase changes indicate a rather small transformation, and the identification of the light chains, in gel electrophoretograms obtained without Na dodecyl-SO₄, in terms of the by now well-characterized subunits, is ambiguous. Correlation between physiological transformation and a change in the myosin light chain pattern has already been reported in rats (Sréter
et al., 1974) and cats (Weeds et al., 1974). The results obtained to date have left open some questions concerning the completeness of the transformation upon cross union of the nerves. Since the resolution of the myosin light chain pattern is most successful in rabbit muscle, it was, therefore, decided to undertake a series of cross-reinnervation experiments in rabbits to investigate in more detail the effect on fragmented sarcoplasmic reticulum (FSR), myosin ATPase, and light chains. Little use has been made of rabbits for nerve cross-union experiments so it was necessary to make a full investigation of the dynamic properties of the muscles which were subsequently subject to biochemical examination.

**EXPERIMENTAL PROCEDURE**

**Operations**

Cross reinnervations were performed between the soleus (SOL) and extensor digitorum longus (EDL) muscles in the right hind limb of 12-wk-old female Californian rabbits. The nerve to the m. anterior tibialis was cut and reflected in order to prevent “fast”-type reinnervation of the EDL (Close, 1969). In some animals the nerve to the SOL or EDL muscle to the contralateral limb was cut and then self-united.

**Dissection of Muscles**

After approximately 11 mo the dynamic properties of the muscles were examined. The animals were anesthetized with Nembutal. The sciatic and common peroneal nerves were separated and cut as high as possible; with the exception of the muscles under investigation the nerves to all other muscles were cut. The EDL and SOL muscles were dissected free leaving their attachments through the proximal tendons intact, and care being taken to preserve the region of nerve entry and the blood supply; however, in several animals the distal venous drainage of the SOL muscle was ligated. The skin around the lower leg was drawn up to contain a pool of liquid paraffin maintained between 36.5 and 37.5°C during the experiment by means of a heating lamp. The proximal tendon of the muscle was clamped with a large pair of Spencer Wells forceps. The distal tendon was tied to a chain connecting it with the transducer system. For recording isometric contractions the muscles were held at optimum length for the isometric twitch, which was also the initial length for isotonic contractions.

**Apparatus and Physiological Measurements**

The chain tied to the muscle tendon ran around the outside of a light-weight aluminum wheel. For isometric recording a peg could be inserted into this wheel so that the muscle pulled directly against a Statham Universal transducing cell, type UC3, (Statham Instruments, Inc., Oxnard, Calif.), in combination with an appropriate load cell accessory, type UL4. Compliance of this system was 0.33 mm/kg. Isotonic contractions were recorded with a Schaevitz Rotary Variable Differential Transformer (Birdsall et al., 1971). This formed the spindle about which the wheel could
rotate. Loads were applied to the muscle by hanging weights on a horizontally positioned second-order lever with a ratio of 11:1. The end of this lever was connected to a chain running over a small wheel concentric with the spindle of the rotary transformer. This further reduced the load applied to the muscle in the ratio 3.65:1, giving a total ratio of approximately 40:1. The transducer assembly and tendon clamp were connected by magnetic bases to a heavy steel table. All stimulus patterns and analysis of contractions were performed by an on-line digital computer (DEC PDP 8/1 and AX08, Distal Equipment, Inc., Maynard, Mass.). The details of this system and the necessary programs were described earlier (Ranatunga, 1972 a, b). Stimulus frequencies for tetanic contractions were: N (normal)-EDL and X (cross-reinnervated)-SOL 250 Hz (isometric) and 400 Hz (isotonic); N-SOL 200 Hz and 250 Hz; X-EDL 125 Hz and 200 Hz, respectively.

At the end of the experiment the muscles were removed, cleaned of oil, blood, and excess tendon, and weighed. A small strip of muscle was cut off and fixed in formol saline for subsequent determination of sarcomere number (Luff, 1975). The bulk of the muscle was reweighed, immediately frozen in liquid nitrogen, and stored at $-85^\circ$.

Biochemical Studies

For biochemical studies the frozen muscles were thawed in isotonic NaCl solution, cleaned of blood and connective tissue, and cut into small pieces with fine scissors for homogenization by a Polytron (Kinematika GMBH, Luzern, Switzerland). The sample was then homogenized for 5 s at 30-s intervals three times. The myofibrils were sedimented by centrifugation for 15 min at 6,000 g and the supernatant was further centrifuged at 40,000 for 50 min in a Sorvall preparative centrifuge (Dupont Instruments, Sorvall Operations, Newtown, Conn.). The fractions sedimenting between 6,000 and 40,000 g were homogenized in a solution containing 0.6 M KCl, 10 mM Tris maleate (pH 7.0) and centrifuged again for 20 min at 40,000 g. The pellet (fragmented sarcoplasmic reticulum, FSR) was rehomogenized in the original volume of a solution containing 0.1 M KCl and 10 mM Tris maleate, pH 7.0.

Myosin was extracted from the sedimented myofibrils with 5 vol of cold KCl-potassium phosphate, pH 6.5 (0.3 M KCl, 0.1 M KH$_2$PO$_4$, 5 mM EDTA, and 5 mM ATP) for 10 min and further purified essentially as described earlier (Sarkar et al., 1971).

The determination of the initial rate of Ca$^{2+}$ uptake by FSR, 0.5 mg of protein/ml for slow and 0.2 mg/ml for fast muscle FSR, was made in a medium containing 10 mM Tris maleate, 20 mM KCl, 5 mM MgCl$_2$, 5 mM K$_2$C$_4$O$_7$, 0.11 mM EGTA, 5 mM ATP, and 0.1 mM $^{45}$CaCl$_2$ under constant stirring. After 1 min a 1-ml portion was removed and filtered through a Millipore filter (HA 0.45-μm filter and AP2002500 prefilter). The reaction was started by adding ATP and the samples were immediately filtered and removed at 3 and 20 s. For simultaneous determination of the ATPase activity and total Ca$^{2+}$ accumulation by FSR (0.05 mg of protein/ml for slow and 0.02 mg/ml for fast) samples were withdrawn at 1, 2, and 5 min for muscle ATPase assay and at 15 min for Millipore filtration (Ca$^{2+}$ uptake).

Ca$^{2+}$-activated ATPase of myosin was measured in a medium containing 0.05 M
Tris HCl (pH 7.6), 10 mM CaCl₂, 2.5 mM ATP, and 0.2 mg of protein/ml. For K⁺-activated ATPase Ca²⁺ was replaced with 5 mM EDTA and 0.6 M KCl. The reaction volume was 2 ml with 2-min incubation at 25°. In the experiments with preincubation at pH 9.2 solutions containing 0.2 mg of protein/ml, 25 mM KCl, 10 mM Tris, 10 mM CaCl₂ were kept at 25° for 10 min. At the end of this period the pH was adjusted to 7.6 by adding Tris to a final concentration of 50 mM and HCl as required. The ATPase assay was then started by adding 2.5 mM ATP.

Protein concentration was determined by a microbiuret method (Itzhaki and Gill, 1964). Na dodecyl-SO₄ gel electrophoresis was carried out as reported earlier (Sarkar et al., 1971) except that 100-mm gels were used in 115-120 X 5-mm glass tubes.

RESULTS

With the operative precautions described under Experimental Procedure to prevent reinnervation with the original nerve, in no case of cross-reinnervation was there any response on stimulation of the original nerve. Therefore, all the muscles can be considered pure in terms of the innervation they received.

Some or all dynamic properties were examined in the muscles of nine rabbits. One animal died during the dissection but it was established that X-SOL and X-EDL were innervated by a single (crossed) nerve and these muscles were, therefore, included in the biochemical analysis. The cross-reinnervated muscles of one rabbit were found to be denervated and were rejected.

Physiologically, the most outstanding feature of these results is the completeness of the transformation of the cross-reinnervated muscles. This is best demonstrated in Fig. 1 where the time-course of the isometric twitch of the N-EDL and X-SOL muscles is indistinguishable as are those of the X-EDL and N-SOL, the latter only differing in having a slightly longer relaxation phase. These results should be compared with previous results from the rat (Close, 1969) and cat (Luff, 1975) showing that the transformation of the isometric twitch was incomplete.

The completeness of the transformation of rabbit muscles by cross-reinnervation is also shown by the ratio of twitch to tetanic tension, which is

\[ \frac{a}{P_o} \]

for X-SOL, although increased in comparison with that for N-SOL, still fell short of that for N-EDL (Table I).
Figure 1. Typical records of the isometric twitch of a normal (N) and cross-reinnervated (X) soleus (SOL) and extensor digitorum longus (EDL) muscles from one experiment. The number to the left of each record is the active tension in newtons (N). The time bar represents 70 ms. All records were made at between 36.5 and 37.5°C with the muscle held at optimum length for the twitch. This animal was examined 341 days postoperatively.

Figure 2. Mean force:velocity curves for the normal (N), self-reinnervated (S) and cross-reinnervated (X) soleus (SOL) and extensor digitorum longus (EDL) muscles. The ordinate is the maximum speed of sarcomere shortening and the abscissa is the isotonic load (P) as a fraction of the maximum isometric tetanic tension (P0). The curves have been drawn using average values for the constants in Hill's equation (Hill, 1938). These results are summarized in Table I.
characteristically low in N-EDL and X-SOL. Even more interesting is the behavior of the shortening velocity in this species. As pointed out previously (Close, 1969; Luff, 1975), the shortening velocity of the whole fiber is an inadequate measure since it depends on the number of sarcomeres in series. A more reliable measure is the intrinsic speed of shortening, viz. the maximum speed of shortening (at zero load) per sarcomere. This speed in rabbit X-SOL is almost the same as that of N-EDL. Interestingly, there occurs an increase in the number of sarcomeres per fiber (Table I), the effect of which is to make the whole fiber speed of shortening greater than that of N-EDL.

Although the shortening velocities of various types of muscle were determined at different optimal stimulation frequencies (see Experimental Procedures), this is not likely to have resulted in artifacts. For, as control experiments (not presented) show, at the stimulation frequency of 400 Hz used for X-SOL both the transformed and, if any remained, slow fibers would be shortening at their respective $V_{max}$. At 200 Hz, used for X-EDL, slow fibers resulting from transformation are fully activated, and any possible remaining

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**TABLE I**

MEASUREMENTS ON NORMAL (N), SELF-REINNERVATED (S), AND CROSS-REINNERVATED (X) SOLEUS AND EXTENSOR DIGITORUM LONGUS RABBIT MUSCLE

| Muscle   | $T_c$ | $P_t$ | $P_o$ | $P_t/P_o$ | $S_n$ | $V_{f,max}$ | $V_{s,max}$ | $a/P_o$ | Mass  |
|----------|-------|-------|-------|-----------|-------|-------------|-------------|---------|-------|
|          |       | N     | N     |           |       |             |             |         |       |
| 6 X-SOL  | 24.1* | ±1.6  | ±0.20 | ±3.0      | 0.127*| 9,180*      | 361.0*      | ±3.21   | ±0.048| ±0.24 |
| (9)      |       |       |       |           |       |             |             |         |       |
| 3 S-SOL  | 64.0  | ±12.3 | ±0.09 | ±3.1      | 0.164 | 6,920       | 131.8       | ±25.7   | ±0.036| ±0.62 |
| (2)      |       |       |       |           |       |             |             |         |       |
| 4 N-SOL  | 60.9  | ±7.2  | ±0.98 | ±3.6      | 0.171 | 6,900       | 120.0       | ±28.3   | ±0.041| ±0.43 |
| (6)      |       |       |       |           |       |             |             |         |       |
| 3 X-EDL  | 53.7* | ±5.8  | ±0.62 | ±2.8      | 0.220*| 5,370*      | 114.8*      | ±17.8   | ±1.26 | ±0.035| ±0.41 |
| (8)      |       |       |       |           |       |             |             |         |       |
| 2 S-EDL  | 21.1  | ±2.3  | ±0.01 | ±1.3      | 0.124 | 6,640       | 298.1       | ±46.02  | ±0.273| 2.43* |
| (2)      |       |       |       |           |       |             |             |         |       |
| 1 N-EDL  | 23.9  | ±2.6  | ±1.68 | ±15.5     | 0.137 | 6,640       | 266.7       | ±40.24  | ±0.296| 3.77  |
| (3)      |       |       |       |           |       |             |             |         |       |

* Probability values for the differences between means have been determined from $t$ values and those means with an asterisk indicate that the difference between means for operated and normal homologous muscles is significant at a probability greater than 0.95.

All records of contraction were made with the muscles between 36.5 and 37.5°C.

The abbreviations above the columns are isometric twitch time to peak ($T_c$); isometric twitch ($P_t$) and tetanic ($P_o$) tension; twitch/tetanus ratio ($P_t/P_o$); sarcomere number ($S_n$); the estimated maximum speed of shortening of whole muscle fibres ($V_{f,max}$) and sarcomeres ($V_{s,max}$) at zero load and the constant ($a$) of Hill’s equation (Hill, 1938).

All measurements of contraction were made with the muscle held at the optimum length for the isometric twitch.

The muscles were examined about 340 days postoperatively.

Numbers in parentheses indicate the number of animals used.

Results are given as mean values ± SD.
fast fibers would still contract at more than 90% of $V_{\text{max}}$. Hence no significant deviation of the measured $V_{\text{max}}$ from the true values is to be expected.

The X-EDL show very considerable atrophy in these experiments. The isometric tetanic tensions were very low in comparison with either the normal or self-reinnervated EDL (Table I). In all cases the muscles were packed with a considerable amount of fat. This again demonstrates the apparent poor compatibility between the soleus nerve and EDL muscle that has been seen before for both the rat (Close, 1969) and the cat (Luff, 1975).

Sarcoplasmic Reticulum

The initial uptake rate of calcium of the cross-reinnervated EDL decreased to the value characteristic of soleus while the rate for cross-reinnervated soleus increased but did not reach the level found in the contralateral EDL (Table II). The same pattern can be seen in the total calcium uptake by FSR, in that the uptake of the cross-reinnervated EDL decreased to a value essentially indistinguishable from that of soleus while the increased value obtained in cross-reinnervated soleus was clearly below the normal values for EDL.

**Myosin**

The ATPase activity of the myosin from cross-reinnervated EDL decreased to a value intermediate between that of control EDL and soleus (Table III). While the ATPase activity of the cross-reinnervated soleus did not quite reach that of the control EDL, it seems that the change that accompanies the transformation of slow to fast muscle was greater than in the fast to slow process. Interestingly, the decrease of ATPase activity after exposure to
pH 9 (alkali lability) characteristic of myosin from slow twitch muscles was not found in cross-reinnervated EDL. This suggests that with respect to this parameter myosin in cross-reinnervated fast muscle is different from that of normal slow muscle. The light chain pattern has proved itself to be a useful discriminator of various types of myosin. The light chain pattern of cross-

**TABLE III**

**EFFECT OF CROSS-REINNervation ON THE ATPase ACTIVITY OF MYOSIN**

| Activator                  | With preincubation at pH 9.2 | μmol mg⁻¹ min⁻¹ |
|----------------------------|-------------------------------|-----------------|
|                            | Activator Without preincubation |                 |
| Normal EDL (7)             | 0.66±0.01                     | 0.65±0.01       | 1.95±0.28  |
| Self-reinnervated EDL (2)  | 0.56±0.04                     | 0.59±0.02       | 2.2±0.002  |
| Cross-reinnervated EDL (9) | 0.31±0.05                     | 0.30±0.09       | 1.36±0.05  |
| Normal soleus (6)          | 0.17±0.001                    | 0.05±0.0004     | 0.82±0.07  |
| Self-reinnervated soleus (2)| 0.19±0.002                  | 0.05±0.001      | 0.98±0.01  |
| Cross-reinnervated soleus (10)| 0.50±0.04                | 0.49±0.06       | 1.63±0.38  |

Numbers in parentheses indicate the number of animals used.
Results are given as mean ± SD.
For details of assays see Experimental Procedure.

**Figure 3.** Polyacrylamide gel electrophoretic pattern in the presence of Na-dodecyl-SO₄ of light chains of myosin from normal, self-reinnervated, and cross-reinnervated muscles. The amount of protein applied to each gel was 30 μg. (a) control SOL; (b) self-reinnervated SOL; (c) cross-reinnervated SOL; (d) control EDL; (e) self-reinnervated EDL; (f) cross-reinnervated EDL. Migration is from top to bottom. The next to the fastest band in c, and to a lesser extent in f, is an oxidized form of the fast type LC₂. The two forms of the latter are indicated by arrows. For details see Experimental Procedures.
reinnervated muscles essentially agrees with the myosin ATPase results in that neither type is completely transformed into the other; the light chain pattern of either type of cross-reinnervated muscle contains the light chains characteristic of both types (Fig. 3). Thus cross-reinnervated soleus contains LC3, the light chain found only in fast muscle, in addition to the LC1 and LC2 chains of both types. Similarly, cross-reinnervated EDL, while retaining the three light chains characteristic of myosin from fast twitch muscle, contains the LC1 and LC2 found in slow muscle myosin. As the studies described above have also been carried out on self-reinnervated muscles of either type, it is clear from our data that this self-reinnervation does not produce the changes seen after cross-reinnervation; this would exclude transient denervation as the cause of the changes seen after cross-reinnervation.

**DISCUSSION**

This is the first detailed report dealing with the effect of cross-innervation on the physiological and biochemical properties of rabbit muscle. This investigation assumes added significance in view of the fact that most of the detailed biochemical work on the properties of myofibrillar proteins and sarcoplasmic reticulum has been carried out on rabbit muscle. The results presented above show an essentially complete transformation after cross-reinnervation with respect to contraction time, intrinsic shortening velocity, and twitch/tetanus tension ratio. Comparison with physiological data on cross-reinnervated muscles in cats and rats indicates that the rabbit is an exceedingly suitable animal for these studies and that the transformation in other animals is in many instances less than complete. It should, however, be pointed out that the only parameter that shows less than complete transformation for X-SOL is $a/P_0$ obtained from force velocity curves. Close (1969) has pointed out that $a/P_0$, which is inversely related to the curvature of the force-velocity curve, is lower in slow twitch muscles than in fast ones. The $a/P_0$ value for X-SOL is significantly lower than that for normal EDL but the value of $a/P_0$ for X-EDL agrees with that for N-SOL. The significance of this finding in terms of biochemical parameters to be discussed below is not clear. The fact that the data for cross-reinnervated muscles could be fitted with Hill’s equation supports the view that there was a homogeneous fiber population after cross-reinnervation. This is in contrast with the situation encountered in X-SOL of the rat (Luff, 1975).

The transformation of the biochemical properties does not parallel in every respect that of the physiological ones. Thus, although the Ca$^{++}$ uptake of the sarcoplasmic reticulum of cross-reinnervated EDL is essentially identical with that of normal soleus, that of cross-reinnervated soleus is considerably lower than that of self-reinnervated EDL. On the other hand, while the myosin ATPase of cross-reinnervated soleus falls short of that in normal EDL, the
X-EDL ATPase activity is considerably higher than that of N-SOL. The alkali lability characteristic of myosin ATPase of normal soleus is absent in X-EDL. The latter fact suggests that cross-reinnervation produces biochemical entities that are different from those found in normal muscle.

It appears that less than full biochemical transformation can produce an essentially complete physiological change from the slow to fast type and vice versa. This is reflected in both time-course of the onset and decay of the isometric tetanus (Sréter et al., 1973, 1974), probably governed by the release and uptake of Ca\(^{2+}\) by the SR, and in the intrinsic velocity of contraction, likely to reflect the properties of the components of the contractile apparatus, including myosin. Cross-reinnervation of either EDL or soleus produces a typical mixed light chain pattern indicating the presence of both types of myosin. It seems that the mixed character of the myosin in transformed muscle is more strongly reflected in the light chain pattern than in the ATPase activity suggesting that the light chains do not directly determine the enzymatic character of the myosin molecule.

The partial dissociation of physiological and biochemical changes appears clearest in the rabbit of all species so far studied (Barany and Close, 1971; Close, 1969; Buller et al., 1969; Weeds et al., 1974). This is partly due to the fact that the physiological transformation in the rabbit is more complete than that reported in other animals (Buller et al., 1969; Close, 1969; Weeds et al., 1974) and partly due to the fact that the less than complete biochemical transformation reflected in the light chain pattern is most clearly seen in the case of the rabbit where the light chain pattern is considerably simpler, particularly for soleus, than that reported for the cat by Weeds et al. (1974). They have stressed the strong correlation between biochemical and physiological changes occurring upon cross-reinnervation and have drawn definite conclusions regarding the neural influence on the phenotype of myosin. Our results raise several questions concerning the fullness of the effect induced by cross-reinnervation. The differences reported in this paper between the extent of physiological and biochemical transformation may be explained by assuming that myosin molecules corresponding to the original muscle type still present in the cross-reinnervated muscle do not make a functional contribution. As far as the SR is concerned, in the case of cross-reinnervated soleus the incomplete change in Ca uptake may be sufficient to bring about the full change in the time-course of the isometric twitch.

In contrast, as we have reported elsewhere, chronic stimulation for less than 15 wk of a nerve innervating a fast muscle with a stimulus pattern simulating that of a slow twitch muscle, viz. 8–10 Hz/s, will completely transform the fast muscle into a slow type as judged by the following criteria: activities of FSR and myosin, alkali lability of myosin, light chain complement, light meromyosin paracrystal staining pattern, intrinsic speed of shortening, and isometric twitch contraction time. The relative roles played by direct nervous
influence and the activity pattern of the muscle itself will require further
careful examination. Since stimulation alone without nerve crossing, as
compared with no stimulation, in these experiments seems to produce a
more profound change in the biochemical properties of muscle, one cannot
exclude the possibility that the changes after cross-reinnervation are due to
a change in the activity pattern whose effect, however, is less far-reaching in
terms of some biochemical changes than that imposed by electrical stimula-
tion. Experiments in which cross-reinnervation is combined with super-
imposed stimulation are needed to throw more light on this problem.

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REFERENCES

BARÁNY, M., and R. I. close. 1971. The transformation of myosin in cross-innervated rat
muscles. J. Physiol. (Lond.). 213:455–474.

BIRDSALL, D. L., A. J. BULLER, and C. J. C. KEAN. 1971. A displacement transducer for meas-
uring muscle shortening. J. Physiol. (Lond.). 213:11P.

BULLER, A. J., W. F. H. M. MOMPERS, and K. SERAYDARIAN. 1969. Enzymic properties of
myosin in fast and slow twitch muscles of the cat following cross-innervation. J. Physiol.
(Lond.). 205:581–597.

CLOSE, R. 1969. Dynamic properties of fast and slow skeletal muscle of the rat after nerve
cross-union. J. Physiol. (Lond.). 204:331–346.

HILL, A. V. 1938. The heat of shortening and the dynamic constants of muscle. Proc. R. Soc.
Lond. B Biol. Sci. 126:136–195.

ITZHAKI, R. F., and D. M. GILL. 1964. A micro-biuret method for estimating proteins. Anal.
Biochem. 9:401–407.

LUFF, A. R. 1975. Dynamic properties of fast and slow skeletal muscles in the cat and rat
following cross-reinnervation. J. Physiol. (Lond.). 248:83–96.

RANATUNGA, K. W. 1972 a. ISOM: A computer program for on-line recording and analysis
of isometric myogram of skeletal muscle. Comput. Programs Biomed. 2:99–106.

RANATUNGA, K. W. 1972 b. Use of a Lab-8 computer for on-line analysis of force velocity
characteristics of skeletal muscle. Comput. Programs Biomed. 2:107–110.

SAMAJA, F. J., L. GUTH, and R. W. ALBERS. 1970. The neural regulation of gene expression
in the muscle cell. Exp. Neurol. 27:276–282.

SARKAR, S., F. A. SÉRÉTER, and J. GERGELY. 1971. Light chains of myosins from white, red
and cardiac muscles. Proc. Natl. Acad. Sci. (U.S.A.). 68:946–950.

SÉRÉTER, F. A. 1974. Transformation of fast myosin into slow type by changed activity pattern.
FEBS (Fed. Eur. Biochem. Soc.) Proc. Meet. 9:18. (Abstr.)

SÉRÉTER, F. A., J. GERGELY, and A. L. LUFF. 1974. The effect of cross-reinnervation on the
synthesis of myosin light chains. Biochim. Biophys. Res. Commun. 56:84–89.

SÉRÉTER, F. A., F. C. A. ROMANUL, S. SALMONS, and J. GERGELY. 1974. The effect of a changed
pattern of activity on some biochemical characteristics of muscle. In Exploratory Concepts
in Muscular Dystrophy II. Excerpta Medica International Congress Series #333. Amster-
dam. 338–343.

SÉRÉTER, F. A., S. SALMONS, F. ROMANUL, and J. GERGELY. 1973. Synthesis by fast muscle of
myosin light chains characteristic of slow muscle in response to long term stimulation. Nat
New Biol. 241:17–19.

WEEDS, A. G., D. R. TRENTHAM, C. J. C. KEAN, and A. J. BULLER. 1974. Myosin from cross-
reinnervated cat muscles. Nature (Lond.). 247:135–139.