Nonlinear Summation of Contractions in Cat Muscles

I. Early Depression

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ABSTRACT Nerves to fast- and slow-twitch cat muscles were stimulated with various numbers of supramaximal pulses under isometric conditions. By subtracting the force produced by \( j - 1 \) pulses from that produced by \( j \) pulses, the contribution of the \( j \)th pulse could be compared with the response to one pulse (twitch response). A less-than-linear summation (depression) was observed during the rising phase of the twitch. This depression became increasingly prominent and longer in duration with repetitive stimulation. A more-than-linear summation (facilitation) was observed during the falling phase of the twitch, which became increasingly delayed and smaller in amplitude with repetitive stimulation. The early depression could be abolished for the first few pulses by Dantrolene [1-(5-p-nitrophenyl) furfurilidene amino hydantoin sodium hydrate], which reduces Ca++ release from the sarcoplasmic reticulum. The depression was less prominent at short muscle lengths or with stimulation of single motor units. A first-order, saturable reaction such as Ca++ binding to troponin or actin binding to myosin can quantitatively account for the early depression.

INTRODUCTION

Repetitive stimulation of skeletal muscles produces contractions that sum nonlinearly. The most obvious example of nonlinear summation is the saturation of tension at the tetanic level with high stimulus rates. The curve of force versus stimulation rate under isometric conditions tends to be sigmoid in shape (Cooper and Eccles, 1930; Mannard and Stein, 1973), indicating a second nonlinearity at low stimulus rates. Cooper and Eccles (1930) also found that the response to two closely spaced stimuli could be considerably larger and more prolonged than expected from the twitch. At low stimulus rates that produce unfused twitches, positive and negative staircase phenomena have been described, which imply a facilitation or depression of the responses to successive stimuli (reviewed by Colomo and Rocchi [1965]). There is also a posttetanic potentiation of mammalian muscle twitches (Brown and von...
Euler, 1938; Standaert, 1964b; Close and Hoh, 1968; Hoh, 1974) as well as a similar phenomenon at the neuromuscular junction (Rosenthal, 1969; Magleby and Zengel, 1975). The length-tension (Gordon et al., 1966) and force-velocity curves (Fenn and Marsh, 1935; Hill, 1938) of muscle show additional, well-known nonlinearities. Finally, small amplitude nonlinearities have been described (Hill, 1968; Joyce et al., 1969; Flitney and Hirst, 1978) that are associated with bending of bonds between myofilaments until they break.

Despite these many examples of nonlinear summation, muscles respond in a surprisingly linear manner to random patterns of stimulation at rates which produce partially fused contractions (Mannard and Stein, 1973; Bawa et al., 1976a; Bawa and Stein, 1976). The responses of muscle under both isometric and nonisometric conditions could be well described by a second-order linear transfer function (Bawa et al., 1976b). However, the best-fitting parameters of the transfer function varied with muscle length and stimulation rate, which implied the presence of underlying nonlinearities (Bawa et al., 1976a). We have therefore searched more systematically for nonlinearities which have a functional importance in contractions of mammalian skeletal muscles.

Much recent physiological work has been done on single muscle fibers of the frog (e.g., Huxley [1974]; Ford et al. [1977]) or giant fibers of the barnacle (Ashley, 1978). Studies on the role of muscles in the control of movement have largely involved mammalian preparations. Since single fibers are much more difficult to dissect, these studies have analyzed the properties of motor units (Burke et al., 1976; Zajac and Young, 1980) or whole muscles (Stein, 1974; Stein and Parmiggiani, 1979). Most mammalian muscles contain a mixture of fast- and slow-twitch muscle fibers. To compare these fiber types and to check the generality of our results, we have studied two muscles in the cat (soleus, a purely slow-twitch muscle [McPhedran et al., 1965] and plantaris, a mixed muscle with a relatively high fraction [about three-fourths] fast-twitch fibers [Ariano et al., 1973]). The muscles were studied in situ so that any nonlinearities observed could not be attributed to isolation from a blood supply or extensive dissection.

This paper concentrates on the less-than-linear summation or early depression that occurs when one or more stimulus pulses are applied during the early portion of a twitch contraction; the facilitation that occurs later in the twitch will be considered in the accompanying paper (Parmiggiani and Stein, 1981). We have analyzed the effects of the number of stimuli, the intervals between stimuli, the amount of Ca++ released, and muscle length on the early depression. The early depression can be accounted for by a first-order saturable reaction such as the binding of Ca++ to troponin or the binding of actin to myosin. A brief description of some of these results has been presented elsewhere (Parmiggiani and Stein, 1979).

METHODS

20 cats were prepared under Nembutal anesthesia for recording from soleus and/or plantaris muscles. The nerves to these muscles were dissected from the point of insertion into the muscles to the point where they join the main sciatic nerve (for
soleus nerve, this involved dissection through the lateral gastrocnemius muscle). The muscles were freed as much as possible from nearby muscles and connective tissue without compromising their blood supply. Each muscle was attached in turn either to an isometric force transducer (FT-10; Grass Instrument Co., Quincy, Mass.) or similar strain gauges. Muscle length was adjusted to the value which gave the maximum twitch tension. All measurements were made at this optimal length, except when length was being varied systematically.

Muscle electrical activity (EMG) was recorded using fine, bare silver wires inserted into each muscle, or with EMG probes sewn to the surface of the muscles (Stein et al., 1977). In some experiments, the L7 and S1 ventral roots were exposed through a conventional laminectomy. Either the muscle nerves, decentralized ventral roots, or filaments dissected from the roots could be stimulated. In these experiments, muscles other than soleus and plantaris in the leg were denervated and the dorsal and ventral roots from L6-S2 were cut. The temperature of the body and muscles was monitored and maintained at 35–37°C. All signals were led to a computer (PDP 11-34; Digital Equipment Corp., Maynard, Mass.) for on-line signal averaging and further processing. Digital filtering using a five-point running mean could also be performed. Digital circuitry (Stein, 1968) permitted the computer to be triggered with a variable delay before the time of stimulation, which permitted flexibility in the timing of pulse trains.

RESULTS

Fig. 1 A shows the response of soleus muscle in the cat to one and two stimuli applied to its muscle nerve. If the contractions summed linearly, the extra tension contributed by the second stimulus should be equal in amplitude and identical in time-course to that produced by the first stimulus (the twitch response). It has long been known for mammalian muscles (Cooper and Eccles, 1930) that the tension contributed by the second stimulus is both larger and longer-lasting than the twitch. To demonstrate this more clearly, the response to one stimulus has been subtracted from the response to two stimuli and the result has been shifted left by the stimulus interval. The contributions of the first and second stimuli can then be directly compared, as shown in Fig. 1 B. The tension contributions are not equal, as would be true in a linear system, since the contribution of the second stimulus in Fig. 1 B has a peak 58% larger than that of the twitch. In addition, the contraction time is 100% longer, and the area contributed by the second stimulus (the integral of force over time) is 137% greater than that of the twitch.

Despite the larger, longer response to the second stimulus, the contribution of the second stimulus initially rises more slowly than the twitch and does not exceed it until about the time of the peak in the twitch tension. Thus, there is an early depression (indicated by the crosshatched area) in the summation of contractile responses which gives way to a later facilitation. In using the terms "depression" (or "facilitation"), we merely refer to a less-than-linear (or more-than-linear) summation of contractile responses recorded at the strain gauge in response to an extra stimulus. Nothing is implied about the forces generated internally or the mechanism involved. The contribution of one stimulus can be subtracted from the contribution of the second stimulus, as shown in Fig.
1C where the negative area (again, crosshatched) represents less-than-linear summation and the positive area represents more-than-linear summation. Since the displays in Fig. 1B and C are equivalent, we will only show the contributions in subsequent figures.

Although the large, later facilitation has been studied in detail (Burke et al., 1976; Ranatunga, 1978; Zajac and Young, 1980), the early depression

Figure 1. (A) Contractile responses of cat soleus muscle to one and two stimuli with an interval of 10 ms. (B) The contribution of the second stimulus (C2) is obtained by subtracting the response to one stimulus from that to two stimuli. The contribution of the second stimulus has been shifted left by 10 ms for comparison with the twitch response (C1). Note that the contribution of the second stimulus initially rises more slowly than the twitch (crosshatched area) before crossing at about the peak of the twitch. (C) By taking the difference between the contributions of the two stimuli, the degree of nonlinear summation can be measured. The negative crosshatched area represents less-than-linear summation (early depression) and the positive area represents more-than-linear summation (later facilitation).
with two stimuli was only recently noted in a brief communication (Parmigiani and Stein, 1979) and has not previously been thoroughly studied. The depression is small (the crosshatched area is only 5.6% of the twitch), but was regularly seen in a variety of muscles. In Fig. 2, a comparison of the responses to one to four stimuli in (Fig. 2A) a purely slow-twitch muscle (soleus) and (Fig. 2B) a largely fast-twitch muscle (plantaris). Although there are differences in the time scales for fast and slow muscles, the patterns of summation

Figure 2. From one to four stimuli were applied to the nerves of soleus and plantaris muscles of the cat. Stimulus intervals of 10 ms (soleus) and 5 ms (plantaris) were used because of the different contractile speeds of the two muscles (note differences in time scales). Five responses were averaged for each stimulus condition and then superimposed. The separation between adjacent traces represents the contribution of each additional stimulus, and these contributions (C1–C4) are plotted in the lower portions of this figure. The contribution of each stimulus has been shifted so that the times of stimulation line up. The separation between the responses to one and two stimuli is greatest for both muscles, which means that the contribution of the second stimulus is largest. However, there is a less-than-linear summation (early depression) in response to the second stimulus which becomes longer and more pronounced for later stimuli.

are similar. This is seen more clearly in the lower portions of Fig. 2 in which the response to \( j - 1 \) stimuli has been subtracted from the response to \( j \) stimuli to determine the contribution of the \( j \)th stimulus.

In both muscles, the contribution of the second stimulus initially rises more slowly (early depression) before crossing the twitch waveform to give a later facilitation. The tension contributions of the third and fourth stimuli in each muscle rise even more slowly and cross the twitch waveform at a later point in time, so the early depression becomes more marked with successive stimuli. By the fourth stimulus, the additional tension integrated over time for cat
soleus was less than the twitch (the early depression had a greater magnitude than the later facilitation). The same trends were observed in all muscles studied, despite some quantitative variation.

**Neuromuscular Transmission**

Fig. 3A shows the EMG for one to four stimuli at 10-ms intervals in a soleus muscle. A subtraction procedure similar to that for tensions was used to obtain the contribution of each stimulus to the gross EMG (Fig. 3B). At very short

![Figure 3](image_url)

**Figure 3.** (A) Surface EMG recorded from a cat soleus muscle with one to four stimuli applied to the nerve at 10-ms intervals. (B) Contribution of each additional stimulus to the total EMG response obtained by subtraction, as for the tension traces (see Fig. 1). Note the similarity of the gross muscle action potential contributed by each stimulus.
intervals (<5 ms) there was some progressive depression of neuromuscular transmission, but at the interval shown in Fig. 3, no depression in the EMG occurred, despite the increasing early depression in the tension production. Careful examination of Fig. 3 B indicates that there was a slight shortening of the time between the early negative peak and the later positive peak, which often occurred in the EMG contributed by later stimuli. This suggests a faster conduction of the signals from one recording electrode to the other. Thus, the early depression cannot be attributed to failure of neuromuscular transmission or other synaptic effects in these muscles. All tension records in this paper were obtained using stimulus intervals where muscle action potentials were not depressed.

**Longer Intervals**

The crossover from the early depression to the later facilitation for the second stimulus typically occurred near the peak of the twitch (see Fig. 2). Thus, one might predict that the early depression would be removed if longer intervals than the twitch contraction time were used (i.e., later stimuli were superimposed on the falling edge of the twitch).

The data shown in Fig. 4 confirm these predictions for the two muscles used. The contributions of the later stimuli are greater than the twitch response at all times and approximately equal to one another. This result was observed for intervals from one to nearly two times the contraction time of

![Figure 4](image-url)

**Figure 4.** Averaged and superimposed contractile responses of (A) soleus and (B) plantaris muscles in the cat. From one to four stimuli were applied to their nerves at intervals of (A) 120 ms, or (B) 30 ms. The response to \( j - 1 \) stimuli was subtracted from the response to \( j \) stimuli to determine the contribution of \( C_j \) of the \( j \)th stimulus, as in Figs. 1-3. With the intervals shown, a facilitation is observed for the contributions of later stimuli (\( C2-C4 \)) at all times compared with the twitch response (\( C1 \)).
each muscle. Thus, the first stimulus switches the muscle into a facilitated state that is maintained for some time. The facilitation is comparable in magnitude to that seen with the short intervals (Fig. 2) so the mechanisms underlying the facilitation have quite a different time-course than those underlying the early depression. In the accompanying paper (Parmiggiani and Stein, 1981) we will examine the time-course of facilitation in detail.

Ca++ Release

The drug Dantrolene, which is used to treat spasticity, specifically reduces release of Ca++ from the sarcoplasmic reticulum (Desmedt and Hainaut, 1977). Fig. 5 shows the summation of force in soleus muscle after intravenous injection of Dantrolene (1.5 mg/kg). The early depression is eliminated for the second and greatly reduced for the third and fourth stimuli. In every experiment in which Dantrolene was tested, the early depression was eliminated for the second and often for the third stimuli. Dantrolene has little effect on tetanic tension (Krarup, 1981).

Figure 5. (A) Total responses of a soleus muscle to one to four stimuli applied at 10-ms intervals. The drug Dantrolene was given intravenously, which reduces release of Ca++ from the sarcoplasmic reticulum. The twitch was reduced to 55% of its control value (not shown) and the area under the twitch to 42% of control. (B) Contributions of each stimulus to the total responses. Note that the early depression is eliminated for the second stimulus and greatly reduced for the third and fourth stimuli (cf. Figs. 1 and 2).
Length

The maximum twitch of soleus muscle in the cat is known to occur at a longer length than the maximum tetanus (Rack and Westbury, 1969), and the twitch-to-tetanus ratio is an increasing function of muscle length. Fig. 6 shows the summation of contractions for stimuli applied at three muscle lengths. The pattern of summation is similar, except that the early depression is not present for the second stimulus at the shortest length shown ($L_0 - 10$ mm).

![Figure 6](image)

**Figure 6.** Effect of muscle length on nonlinear summation in response to stimulation of cat soleus muscle with 1, 2, 3, or 23 pulses at 10-ms intervals. (A) The total responses to 1 and 23 pulses were used to estimate the twitch-to-tetanus ratio. (B) The contributions of the first three pulses ($C1-C3$) were calculated as in previous figures. The early depression with the second stimulus (crosshatched area) decreases as the muscle length is decreased from the optimal length for the twitch ($L_0$) to $L_0 - 5$ mm and disappears at $L_0 - 10$ mm.

The twitch-to-tetanus ratio is smallest at this length (0.14, compared with 0.29 at $L_0$), so the reduction in the early depression is again associated with a reduction in the twitch-to-tetanus ratio.

Motor Units

In a few experiments, the ventral roots were divided to isolate single motor units to soleus muscle. The pattern of summation shown in Fig. 7 is typical of 16 motor units studied. Although the forces are about two orders of magnitude
smaller than for the whole muscle, the pattern of summation is qualitatively similar, except that an early depression is absent for the second stimulus. An early depression with two stimuli was never observed in any motor unit, although the muscle length was varied quite widely for a few of the units. A depression was generally observed with the third stimulus and invariably with the fourth stimulus. The later facilitation was significantly greater for the single units than for the whole muscle (Parmiggiani and Stein, 1981), and the twitch-to-tetanus ratio was significantly smaller. Thus, as in previous sections,

![Graph](image)

**Figure 7.** Total responses (A) and contributions (B) obtained from stimulating a single soleus motor unit with one to four stimuli at 10-ms intervals. The data were processed in the same way as for the whole muscle in Fig. 2. The contribution of the second stimulus (C2) is larger at all times than the twitch (C1), so no early depression is present.

variations that reduce the twitch-to-tetanus ratio tend to reduce or abolish the early depression. The reasons for these results will now be discussed in more detail, and a model which can account quantitatively for the early depression will be proposed.

**DISCUSSION**

We have found two phases of nonlinear summation under a wide range of conditions in fast- and slow-twitch mammalian muscles. An early depression or less-than-linear summation occurs when a second contraction is superimposed on the rising phase of a twitch, and a phase of facilitation or more-than-linear summation is seen later in the time-course of the twitch. The two
phases could be different aspects of the same basic phenomenon since the early depression becomes more prominent with repetitive stimulation as the later facilitation becomes less marked. However, we believe that these two types of nonlinearities arise from distinct mechanisms for several reasons: (a) The later facilitation is still prominent with intervals longer than the contraction time of the muscles studied (Fig. 4), whereas the early depression is completely eliminated. The time-course of these processes is examined in the accompanying paper (Parmiggiani and Stein, 1981). (b) The two processes are pharmacologically distinct in that Dantrolene, which specifically reduces Ca++ release from the sarcoplasmic reticulum (Desmedt and Hainaut, 1977), eliminates the early depression (Fig. 5). (c) A simple formulation can effectively account for the early depression and mathematically explain the separation from the later facilitation (see below: Saturation).

Twitch-to-Tetanus Ratio

All of our experimental manipulations that decreased the twitch-to-tetanus ratio decreased or abolished the early depression. This was true whether the muscle was treated pharmacologically by adding Dantrolene (Fig. 5), mechanically by decreasing muscle length (Fig. 6), or electrically by stimulating single units (Fig. 7) rather than the whole nerve. These results suggest that the early depression arises from a saturable process which becomes increasingly prominent as force levels approach the tetanic value. Before considering this suggestion in more detail, we will discuss two other possible explanations: a progressive failure in neuromuscular transmission or some loss of tension from later stimuli because of viscoelastic properties linking the force generators of muscle to the external recording devices.

Muscle Excitation

The failure of neuromuscular transmission or slowing of muscle impulse conduction is unlikely to account for the early depression, except at the very shortest intervals (<5 ms). Similarly, the backfiring of the motor nerve, which can cause repetitive muscle action potentials under some conditions (Brown and Matthews, 1960; Staendart, 1964a), was not a problem in these experiments. At the intervals used throughout this study, the EMG to later stimuli was virtually identical to that to the first stimulus (Fig. 3). The possibility remains that the muscle action potential at the surface membrane was similar but that spread of the action potential down into the transverse tubules was diminished. Reduced spread of the action potential into the tubules would have little effect on the EMG, but would reduce the release of Ca++. However, a reduced release of Ca++ should produce a uniform decrease in the contraction, rather than a depression limited to the rising phase, followed by a facilitation later in the time-course of the contraction.

Viscoelastic Effects

The elastic properties of muscle are also unlikely to account for early depression. The absence of the early depression, which occurred at short lengths or with single motor units, might be due to the viscoelastic properties. For
example, a reduction of the twitch at short, nearly slack length of the muscle (Fig. 5) could account for the lack of early depression when comparing the second contribution to the reduced twitch. Similarly, the early part of the twitch in a single motor unit from a large muscle may be artificially reduced (Fig. 8) while the motor unit takes up slack in the fine tendinous elements in which it is inserted. This could explain the significantly greater facilitation (i.e., relative to a reduced twitch) observed with single units compared with whole muscles. Previous studies of facilitation of cat motor units (Burke et al., 1976; Zajac and Young, 1980) have not reported an early depression, nor was it observed for the second stimulus in any of our motor units—although a depression was present with later stimuli in a train. Thus, the elastic properties of muscle could account for some reductions in the early depression, but not for the phenomenon itself. The viscous properties of muscle, which are well described by Hill's force-velocity curve (Hill, 1938), might a priori account for some of the early depression. However, the limited amount a muscle can shorten against series elastic elements in the isometric state does not appreciably affect its ability to respond to a second stimulus (R. B. Stein and F. Parmiggiani, unpublished observations).

Saturation

The increasing depression with additional stimuli and the dependence of the depression on the twitch-to-tetanus ratio under a variety of experimental conditions are all consistent with the early depression arising from a saturating process within the muscle. From experiments on amphibian muscle at low temperatures, Hill (1949) suggested that a muscle was maximally activated for some period of time, which he referred to as the "plateau of the active state." This plateau would produce an occlusion of force generation since a later stimulus could not contribute any additional force until the active state fell below its plateau level. A considerable delay in the effects of a second stimulus is observed for frog muscles in the cold (MacPherson and Wilkie, 1954). However, it is generally agreed that mammalian muscles at normal temperatures are not maximally activated by single stimuli (Close, 1972). The contributions of later stimuli in the short trains studied here began to rise with the same delay as the twitch (see Figs. 1, 2, 5, and 6).

Rather than a frank occlusion of force generation, we envisage a gradual saturation as the Ca\(^{++}\) released by successive stimuli occupy more and more of the sites on troponin, for example. This suggestion can be made more precise by considering a reversible reaction in which n molecules of a substance A bind to another molecule B to form a product P = A\(_n\)B (see also Taylor [1969])

\[
\begin{align*}
\text{nA + B} & \underset{k_2}{\underset{k_1}{\rightleftharpoons}} \text{P,} \\
\end{align*}
\]

where \(k_1\) and \(k_2\) are the forward and backward rate constants for the reaction. Then, the change in [P] (square brackets indicate concentrations) will be

\[
\frac{d[P]}{dt} = k_1[A]^n[B] - k_2 [P].
\]
This equation can be solved for the concentration of A by algebraic manipulation

\[
\frac{[A]^n}{K} = \frac{[P] + \tau d[P]/dt}{[B]},
\]

(3)

where \( K = \frac{k_2}{k_1} = [A]^n[B]/[P] \) is the dissociation constant for reaction 1 and \( \tau = 1/k_2 \) is the average lifetime of the product. If we now assume that force \( F \) that the muscle develops is proportional to \( [P] \) and that the maximum or tetanic level of force \( F_m \) is proportional to the maximum concentration \( M \) of product which can be formed, where \( M = [B] + [P] \), then Eq. 3 becomes

\[
\frac{[A]^n}{K} = \frac{F + \tau dF/dt}{F_m - F}.
\]

(4)

The quantities on the right of Eq. 4, \( F, dF/dt, \) and \( F_m \), are all measurable, so the concentration of the reactant A can be easily computed, relative to the dissociation constant \( K \). The absolute values of \( [A] \) and the constant \( K \) may not be known without independent measurements, but the left side of Eq. 4 gives the relative values of these as a dimensionless quantity.

The simplest case occurs when \( n = 1 \) and \( \tau \) is sufficiently small that the differential term can be neglected. Then, the product is short-lived, and force generation is always in equilibrium with \( [A] \). Eq. 4 can be rearranged to give

\[
F = \frac{F_m [A]}{K + [A]}
\]

(5)

which is the well-known Michaelis-Menten equation of enzyme kinetics. Thus, reaction 1 is formally equivalent to a saturating, first-order enzyme reaction. By using Eq. 4 with these simplifying assumptions, the data of Fig. 2A can be transformed to give the apparent concentrations of A at each point in time and the contribution of each stimulus to these concentrations (Fig. 8A).

Note that in performing the transformation illustrated in Fig. 8, we are not trying to model force production. Rather, we are using the experimentally obtained force records to compute the time-course (for several sets of assumptions) that the release of an activator substance should follow to account for the observed force. After the transformation, the increments produced by later stimuli in \( [A] \) (Fig. 8A, upper part) are much more even than the increments in force (Fig. 2A) from which they were computed, and the contributions (Fig. 8A, lower part) virtually superimpose. The early depression has been eliminated in that all contributions rise virtually synchronously. Thus, the early depression can be quantitatively accounted for by the saturation reaction 1.

Thus, under these assumptions the first stimulus would generate a certain concentration of activator A with the time-course shown in Fig. 8A. The first stimulus would also switch the muscle into a facilitated state that is maintained for some time. The early depression in force production (Fig. 2A) would arise from the limited amount of the substance B for A to bind to form the product P necessary for force production. The transformation of Eq. 4 mathematically...
accounts for the separation of the early depression from the later facilitation. Such a separation was possible pharmacologically by using the drug Dantrolene (Fig. 5) and using longer intervals (Fig. 4) for which the saturation at

![Graphs showing concentration changes](image)

**Figure 8.** Estimated concentrations [A] of a substance released by one to four stimuli and the contributions of each stimulus (C1–C4) to the total concentration for three different assumptions (A–C). The data of Fig. 2A for soleus muscle have been transformed using Eq. 4 with different values of \( \tau \) and \( n \). Note that in (A) there is no early depression and the contributions of later stimuli (C2–C4) virtually superimpose. This is true to a lesser extent in (B) but does not occur with the parameters in (B). The results in Fig. 8A suggest that a substance A is released by the first stimulus and that release by later stimuli is facilitated. The early depression in force generation (Fig. 2A) would then result from an increasing lack of molecules for A to bind in order to generate force. These implications are discussed further in the text.

High force levels should be less marked. Although the separation is only shown in Fig. 8A for a single condition, the mathematical transformation produced a similar result at all intervals in both muscles under normal physiological conditions.
The type of reaction responsible for the early depression can be constrained further. For example, Fig. 8 B shows results calculated from Eq. 4 assuming that two molecules of A bind to B (n = 2 and \( \tau = 0 \)). The removal of the early depression and the superimposition of the contributions from later stimuli do not occur. Thus, a second-order reaction would not fully account for the early depression. Other assumptions about the kinetics for release of the activator A would have to be added to account for the residual depression still observed in Fig. 8 B. A similar result is obtained for greater values of n, so the preferable assumption is that the reaction is a first-order, saturable reaction. Only with a first-order reaction does the transformation completely eliminate the early depression and provide a mathematical basis for the separation of the early depression from the later facilitation.

Fig. 8 C shows the effect of changing the time constant \( \tau \) to 40 ms. With the differential term included, the peaks are now correlated more with the rate of change in force rather than its magnitude. Even with this large a time lag, the early depression is still eliminated for the second stimulus and much reduced for later stimuli. The analysis can also provide an upper limit on possible values of \( \tau \). If \( \tau \) is increased sufficiently, the numerator in Eq. 4 becomes negative during the relaxation phase of the twitch, when \( \frac{dF}{dt} \) is negative. Then, a negative value of \([A]\) is predicted, which cannot occur. Increasing the value of \( \tau \) to 50 ms produces a negativity, so the average lifetime of the product P must be <50 ms.

Various candidates for this saturation reaction can be suggested, such as the binding of Ca\(^{++}\) to troponin (Taylor, 1969) or the myosin head to actin. Speculation concerning these or other possible reactions is probably not fruitful until Ca\(^{++}\) transients (Blinks et al., 1978; Eusebi et al., 1980) or myosin binding (Huxley et al., 1980) is measured under comparable conditions.

However, the simple mathematical basis for the separation of the two types of nonlinearities in the summation of muscle twitches should help to distinguish these possibilities experimentally. A better description of the nonlinearities should also be directly applicable to the determination of optimal patterns for activating mammalian muscles (Stein and Parmiggiani, 1979; Zajac and Young, 1980) and the role of muscle properties in the overall control of movement (Stein, 1974).

Helpful comments on the manuscripts were provided by Sir Andrew Huxley, Drs. F. Colomo, D. Morgan, K.G. Pearson, T. Gordon and A.S. French. Dr. French and Mr. R. Rolf provided assistance in developing the computer programs used for analysis.

The research described in this series of papers was supported in part by grants from the Muscular Dystrophy Association of Canada and the Medical Research Council of Canada. F. Parmiggiani was a NATO fellow.

Received for publication 12 November 1980.

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