A QUANTITATIVE MODEL OF ACTIN-MYOSIN INTERACTION IN SKELETAL MUSCLE

MORTON ORENTLICHER, *Department of Neurology, College of Physicians and Surgeons, Columbia University, New York 10032 and*
ALLEN GERSHO, *Bell Telephone Laboratories, Inc., Murray Hill, New Jersey 07974*

ABSTRACT Biochemical schemes for the actomyosin ATPase cycle as well as the cooperative regulation of ATPase activity are incorporated into a model of the contractile process of intact muscle. This model is shown to describe accurately the tension developed by skinned muscle fibers in the absence of Ca. This work adds to the evidence that the extrapolation of results from purified protein systems to intact muscle may be valid. Extensions to the case of Ca-activated tensions are discussed.

INTRODUCTION

Myofilament proteins of skeletal muscle interact in a complex manner so as to maintain a near-zero level of energy use (ATPase rate) at rest, but reach full activation within milliseconds after muscle stimulation. Aspects of the ATPase reaction (Lymn and Taylor, 1971; Eisenberg and Kielley, 1973; Bagshaw and Trentham, 1974) and the regulatory system intrinsic to the myofilaments (Weber and Murray, 1973) have been described for purified protein systems. However, a quantitative description of this regulatory system has not yet appeared, nor has the applicability of these biochemical results to intact muscle been shown. We demonstrate that these biochemical results can be incorporated into a quantitative model of intact muscle, and that this model can explain some recent physiological data. These data have been obtained with skinned muscle fibers, i.e., muscle cells with their surface membranes disrupted or destroyed. We shall focus on a description of the tension produced by these fibers in the absence of calcium (Ca).

Muscle fibers do not require the presence of Ca ions to produce tension if the level of substrate, [MgATP], is sufficiently low. The generation of tension in the absence of Ca was reported by Filo et al. (1965) for glycerinated rabbit psoas muscle and first systematically studied by Rueben et al. (1971), using mechanically skinned crayfish fibers. Reuben et al. (1971) found an increase of tension with increasing substrate concentration at low [MgATP], followed by a decline in tension at superoptimal [MgATP]. This relation between tension and [MgATP] has been confirmed for mechanically disrupted frog skeletal and rat cardiac muscle (Fabiato and Fabiato, 1975) as well as with rabbit psoas (Zollman et al., 1976) and human skeletal muscle (Wood et al., 1975). Studies on mechanically skinned crayfish muscle have demon-

strated a quantitative fit to a "substrate inhibition" model of tension regulation (Reuben et al., 1971; Brandt et al., 1972). However the enzymological model of substrate inhibition is too great a simplification of the biochemical evidence, and a specific inhibitory site at which the substrate might act has not been found (Taylor, 1972). The cooperativity hypothesis of Weber (Weber and Murray, 1973) may be able to describe the "substrate-inhibition" data if it is properly formulated for a myofilament lattice. In this paper we develop such a formulation, and test the ability of this scheme to describe tension as a function of substrate concentration in the absence of Ca. Possible extensions of this scheme to Ca-activated tension are presented in the Discussion.

After a brief review of the biochemical basis for this work, a scheme is presented for incorporation of the biochemical concepts of the ATPase cycle and the cooperative interaction of actin and myosin into a plausible model for intact muscle. This scheme includes an explicit model for the description of mechanical activity of muscle in terms of biochemical rate constants. Although this model is useful for analysis of muscle dynamics, in this paper we limit our discussion to the regulation of isometric tension.

BIOCHEMICAL BACKGROUND

The developments regarding actomyosin ATPase most relevant to this work have been the elucidation of the cyclic interaction of myosin and actin, and the discovery of the regulatory role of myofilament proteins. Unless specifically noted, this work was done with purified proteins or protein fragments, a possible source of significant difference from experimental results obtained under more nearly physiological conditions. These divergencies will be discussed after a review of the recent literature.

The Actomyosin ATPase Cycle

Myosin acts as a catalyst for the hydrolysis of MgATP, but the ATPase rate of myosin in combination with its co-factor, actin, can be orders of magnitude larger than the myosin ATPase rate. Studies on both myosin and actomyosin ATPase from

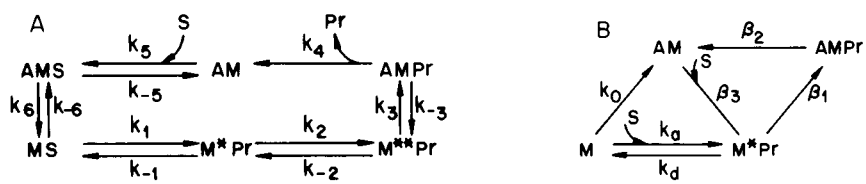

FIGURE 1 Biochemical schemes for the actomyosin ATPase cycle. (*A*) The dominant ATPase pathway of actomyosin, according to Eisenberg and Kielley (1973). Symbols are defined in the text. The step M*Pr → M**Pr has been identified as rate limiting (Eisenberg and Kielley, 1973). Also, at submillimolar levels of [S], step 5 is much slower than step 6. (*B*) A simplified model for the ATPase cycle. Knowledge of slow steps allows elimination of M**Pr, AMS, and MS from the scheme of 1*A*. This leaves the right-hand triangle of states M*Pr, AMPr, and AM. It will be shown that nucleotide-free myosin, M, is crucial for contractile regulation. The pathway for reactions of M is through the left-hand triangle: M*Pr, M, and AM.

the laboratories of Taylor (Lymn and Taylor, 1970, 1971) and Eisenberg (Eisenberg and Kielley, 1973; Mulhern et al., 1975) as well as others have shown that hydrolysis of ATP by actomyosin involves a complex sequence of reactions. The rate-limiting step in this sequence appears to be the transformation of the myosin-ATP complex from a "refractory" state (M*Pr) to a state (M**Pr) where binding of actin (AMPr) may occur (Eisenberg and Kielley, 1973). Their scheme for actomyosin ATPase is presented as Fig. 1 A. (A more detailed scheme has been presented by Bagshaw and Trentham, 1974, and their formalism has been used by Chock et al., 1976, in studies of the refractory state of myosin). The symbols M for myosin and A for actin, as well as superscript * to indicate transient states, are conventional; S indicates the substrate molecule and Pr indicates nucleotide bound to myosin. The rate-limiting step in the Eisenberg scheme is step 2.

Actomyosin Regulation

Our understanding of the regulatory roles of contractile proteins advanced with the isolation of troponin and determination of its role as the Ca-switch in the myofilament system (Ebashi et al., 1968). Further work has demonstrated that the actin monomers that form each strand of the thin filament are organized in seven-monomer units through a tropomyosin molecule that lies alongside each strand in the thin filament, and that each tropomyosin has an associated troponin (Haselgrove, 1973; Huxley, 1973). As the authors cited point out, this provides a mechanism for simultaneous regulation of the cofactor activity of actin monomers.

Working with myofibrils, Weber showed an apparent substrate-inhibition phenomenon for myofibrillar ATPase (Weber, 1969), which was very similar to the relation of tension to substrate concentration of skinned muscle fibers found by Reuben et al. (1971). Moreover, this inhibitory effect of MgATP could be removed by sufficiently high [Ca] (Weber, 1969). These results are consistent with the idea that ATPase and tension of intact systems are correlated, and are similarly regulated. More recent work, predominantly with reconstituted protein systems (Bremel and Weber, 1972; Bremel et al. 1973), has led Weber to formulate a theory of cooperative myosin-actin interaction that can explain both the "substrate inhibition" phenomenon as well as the effects of varying the myosin/actin ratios on ATPase activity. This theory is presented in detail in Weber and Murray (1973).

As we have noted, each thin filament is composed of N member units of actin monomers, where N is probably seven. In the Weber model, all elements of a unit are regulated simultaneously, so that one can refer to the activity level of a unit. Thus each actin monomer of an N-member unit has the same ability to act as cofactor for accelerating the myosin-ATPase rate. Three levels of cofactor activity were proposed (Weber and Murray, 1973): (*a*) Off: no cofactor activity of a unit in the absence of bound Ca or myosin; (*b*) On: a partial activation of a unit when Ca is bound to its troponin; (*c*) Potentiated: maximal cofactor activity of actin units to which myosin has formed "rigor links." Murray (1975) has interpreted his data in terms of a requirement for one rigor link to an actin unit to cause potentiation.

We note at this point that another school of biochemists (Tonomura, 1972) has presented a scheme for the actomyosin ATPase cycle that intrinsically produces a "substrate inhibition" relationship. Reasons for rejecting this scheme has been presented by Taylor (1972), but the dispute continues (Arata et al., 1975).

Limitations of Biochemical Analysis

Before building an analysis of muscle contractility on the basis of these biochemical results, it is necessary to consider their limitations. In addition to expected quantitative changes in the rate constants on going to intact muscle cells, there are also some unexplained deviations from physiological results apparent in the biochemical data.

A major conclusion regarding the ATPase cycle drawn by Eisenberg and Kielley (1973) is that the rate-limiting step for the cycle occurs on the dissociated myosin. In the intact muscle, there is the possibility that tension influences the rate constants; in particular the transitions involving AM may be slowed so that one of them (such as step 4 of Fig. 1 A) may be rate-limiting. (A rigorous method for analysis of the relationship between tension, rate constants, and myofilament geometry has been presented by Hill et al. [1975]). Since the biochemical ATPase measurements are of necessity made in systems without external forces to restrain the rates of molecular transitions, they are more comparable to physiological measurements with freely contracting muscle than to those from muscle held isometric. The biochemical measurement might correlate with cycle rates of unloaded muscle, i.e., at the maximum velocity of shortening, V_{max}. (This was indicated by the correlation of V_{max} with myosin ATPase rate, demonstrated by Bárány [1967].) Similarly, rate constants for steps 5 and 6 may differ between the tension-regulated system of intact muscle and the biochemists' soluble systems. This strain-dependence of rate constants is discussed further in Results. Additionally, the conditions of salt concentration and temperature used in biochemical research typically differ from physiological conditions, and must lead to changes in values for the rate constants.

A striking divergence between physiological and biochemical data appears when one considers the effect of Ca on the ATPase of reconstituted systems and its effect on intact myofibrils. The only published data showing the effect of Ca on the substrate dependence of the ATPase of a reconstituted system over a significant range of [MgATP] (Skikegawa and Tonomura, 1972) demonstrate only a few tenths of a log unit shift in the peak of the ATPase *vs.* [MgATP] curve on going from no Ca to high [Ca] salines. This is consistent with the data from Weber's group (Bremel et al. 1973), in that their Ca-saturated actomyosin ATPase also peaks at low [MgATP]. On the other hand, no peak has been found for the curves with Ca-saturated myofibrils (Weber, 1969; Weber and Murray, 1973). These myofilament data are consistent with skinned fibers, which have shown a shift of three log units, between no Ca and saturating Ca, in the peak of the tension *vs.* [MgATP] relation (Brandt et al., 1972; Orentlicher, unpublished experiments). Thus, one must conclude that reconstitution of the actomyosin regulatory system has not been fully successful (Weber and Murray, 1973).

It is not established that data from purified-protein systems can be used to describe the chemistry of intact muscle. In what follows we present a muscle model based on schemes proposed to describe the purified-protein chemistry. If this model correctly describes the mechanochemistry of intact systems, this would indicate the relevance of the purified-protein chemistry to that of intact muscle.

A MODEL FOR COOPERATIVITY IN MUSCLE CONTRACTION

In this section we construct a model of an intact myofilament lattice that incorporates the concept of *N*-element actin groups potentiated only when at least one rigor link is made to a member of the group. First, Fig. 1 A must be simplified so that a minimal number of parameters characterizes bridge cycling, and biochemical states corresponding to force bonds and rigor bonds are defined. Next, a conceptual model of a myofilament lattice that incorporates these cross-bridge states must be formulated. Lastly, an expression for the tension of such an assembly must be derived. Computations of the substrate dependence of zero-Ca tensions have been made with such a model, and they will be presented in the Results section.

The ATPase Cycle

It has been shown that the dissociation of actomyosin after substrate is bound, AMS → MS, is very rapid (Lymn and Taylor, 1971), and that the initial transformation of substrate by myosin (MS → M*Pr) as well as the combination of myosin-product complex with actin are also fast (Eisenberg and Kielley, 1973), while the transition M*Pr → M**Pr is slow. From these biochemical results it follows that M*Pr is the form of the myosin-nucleotide complex present in highest concentration, and that the concentration of the AMS form of the actomyosin complex must be relatively low. Therefore the scheme of Fig. 1 A can be simplified for the purposes of describing either tension or ATPase data, by deletion of the states AMS, MS, and M**Pr. We use terminology similar to that of Weber and Murray (1973) and denote AMPr as a force bond and AM as a rigor bond. The back reactions in the cycle have also been omitted as negligible. In what follows, [MgATP] will be abbreviated by [S], and effective first-order rate constants for the ATPase cycle in Fig. 1 B have been labeled β_1, β_2, and β_3.

Since the formation of rigor links from myosin free of nucleotide is central to the cooperativity model (Weber and Murray, 1973), an addition is required to Fig. 1 A. The set of possible states must include myosin unassociated with either actin or nucleotide, M. While [M] can be made large in biochemical experiments (Bremel et al., 1973), in the intact muscle [M] must always be small, since the myosin/actin ratio is close to unity and the rate of formation of rigor bonds (M → AM) is very high. (More details will be found in the Discussion.) It will be shown later that the reaction sequence M*Pr → M → AM may be critical for tension regulation, so this loop is included in Fig. 1 B.

Reference to Fig. 1 shows that $\beta_1 = k_2$, $\beta_2 = k_4$, $\beta_3 = k_5[S]$. The other rate constants are k_d, effective rate constant for dissociation of substrate from myosin:

k_a, rate constant for binding MgATP to myosin, k_0, rate constant for binding myosin to actin. Estimates for these constants available from the biochemical literature are employed in Results.

Myofilament Lattice as a Lattice of Cross-Bridges

In contrast to soluble protein systems, the muscle fiber is composed of parallel filaments of actin and myosin, in which each myosin is surrounded by several actins. The geometry of the myofilament lattice requires a detailed description for an exact analysis of the in vivo interactions of actin and myosin. One may think of this lattice as composed of neighboring actin monomer-myosin pairs capable of forming actomyosin cross-bridges, and each pair could be considered a "cross-bridge site." A useful approximation to the myofilament lattice is therefore a lattice of cross-bridge sites in which different states of a site correspond to different stages in the cyclic interaction of a myosin with an actin. By being more structured than the actual myofilament lattice, this approximation eliminates the problem of describing the multiplicity of actins with which a myosin can interact at any time.

Cross-bridge sites are organized into units of N elements by the tropomyosin linkage of actins. These are the cooperative units of the Weber hypothesis (Weber and Murray, 1973). Although sites within a unit are coupled to one another, since they are all part of either a potentiated or nonpotentiated unit, the units are independent of one another. Therefore, the statistics of the units may be described in a straightforward manner and in the following we shall focus on the kinetics of transitions between states of a unit. As suggested by Murray (1975), we take units with no rigor bonds as nonpotentiated, and all other units as potentiated. Note that as long as a unit remains potentiated, the individual sites in that unit independently make the transitions shown in Fig. 1 B. We make use of this property when we compute the transition frequencies between states of a unit.

The next two subsections give a mathematical description of this model.

Statistics of a Lattice of Cooperative Units

The statistics of this model can be derived as follows. The state of an N-member unit (as distinct from the state of an individual cross-bridge site) is defined by four numbers (n_1, n_2, n_3, n_4), where n_1 = number of dissociated cross-bridges with bound nucleotide; n_2 = number of force bonds; n_3 = number of rigor bonds; n_4 = number of dissociated cross-bridges without bound nucleotide; and $n_1 + n_2 + n_3 + n_4 = N$.

State 1 will be referred to as dissociated myosin or dissociated site and state 4 as nucleotide-free myosin. State 2 is the force bond and state 3 is the rigor bond. Each of the n_i can vary from zero to N, where N is believed to be seven.

A cooperative unit makes a transition whenever one of its constituent sites changes its state. Since sites within a potentiated unit make independent transitions, the transition frequency between states of a cooperative unit is calculated by multiplication of the rate constant for a transition by a particular site and the number of sites of that type in the unit. States to which transitions from a potentiated state can be made and the transition frequencies are given in Table I.

TABLE I

POSSIBLE TRANSITIONS OF A POTENTIATED UNIT IN STATE (n_1, n_2, n_3, n_4), $(n_3 \neq 0)$

State to which transition is made	Corresponding site transitions	Transition frequency
$(n_1 - 1, n_2 + 1, n_3, n_4)$	$1 \rightarrow 2$	$n_1 \beta_1$
$(n_1, n_2 - 1, n_3 + 1, n_4)$	$2 \rightarrow 3$	$n_2 \beta_2$
$(n_1 + 1, n_2, n_3, n_4 - 1)$	$4 \rightarrow 1$	$n_4 k_a$
$(n_1 - 1, n_2, n_3, n_4 + 1)$	$1 \rightarrow 4$	$n_1 k_d$
$(n_1, n_2, n_3 + 1, n_4 - 1)$	$4 \rightarrow 3$	$n_4 k_0$
$(n_1 + 1, n_2, n_3 - 1, n_4)$	$3 \rightarrow 1$	$n_3 \beta_3 \quad (n_3 > 1)$
$(n_1 + n_2 - 1, 0, 0, n_4)$	$3 \rightarrow 1, 2 \rightarrow 1$	$\beta_3 \quad (n_3 = 1)$

If a unit is nonpotentiated, it contains only dissociated myosin and nucleotide-free myosin. We assume that force bonds break immediately after the last rigor bond is broken. Just as for potentiated units, sites within a nonpotentiated unit make independent transitions. Therefore transition frequencies are calculated in the same manner. States to which a transition from a nonpotentiated state can be made and the transition frequencies are given in Table II.

There are two transitions that cause a change in potentiation. When the rigor bond of a potentiated unit with only one rigor bond breaks, that unit becomes nonpotentiated: $(n_1, n_2, 1, n_4) \rightarrow (n_1 + n_2 + 1, 0, 0, n_4)$. Thus when the sole rigor bond breaks, the n_2 force bonds also immediately break so that the number of dissociated bonds becomes $n_1 + n_2 + 1$. If a nucleotide-free myosin of a nonpotentiated unit binds to its corresponding actin to form a rigor bond, that unit becomes potentiated: $(n_1, 0, 0, n_4) \rightarrow (n_1, 0, 1, n_4 - 1)$. The steady-state distribution implies an equality in the rates of loss and production of potentiated units.

The system of linear equations formed by setting the net rate of change in concentration of each state to zero can be solved to give the steady-state concentrations for each state of a cooperative unit. The number of states, m, of a cooperative unit is evaluated in Appendix A. By numbering the states of a unit sequentially from 1 to m, labeling the steady-state concentrations (or probabilities) as x_i for the i^{th} state, and denoting the transition frequency from state i to state j as λ_{ij}, we obtain the usual system of equilibrium equations:

TABLE II

POSSIBLE TRANSITIONS OF A NONPOTENTIATED UNIT IN STATE $(n_1, 0, 0, n_4)$

State to which transition is made	Corresponding site transitions	Transition frequency
$(n_1 - 1, 0, 0, n_4 + 1)$	$1 \rightarrow 4$	$n_1 k_d$
$(n_1 + 1, 0, 0, n_4 - 1)$	$4 \rightarrow 1$	$n_4 k_a$
$(n_1, 0, 1, n_4 - 1)$	$4 \rightarrow 3$	$n_4 k_0$

$$\sum_{i=1}^{m} \lambda_{ij} x_i = 0 \quad \text{for } j = 1, 2, \ldots, m, \tag{1}$$

where

$$\lambda_{jj} = -\sum_{i \neq j} \lambda_{ji}.$$

Solution of this system of linear equations gives the exact steady-state concentration of each state of the cooperative unit as modeled above. Details of the method are given in Appendix A. Results of such computations will be given after an approximate method is presented.

Algebraic Description of the Cooperative Model

While the kinetic method just described is useful for computational studies, it cannot lead to explicit algebraic relations for tension or ATPase activity. Since such explicit relationships are heuristically useful, we derive approximate equations based on one assumption: the probability of a site being in a particular state *if it is in a potentiated unit* is given by the probability of that state if all sites were independent. This assumption might introduce serious errors under conditions in which relatively few units are potentiated. However, a comparison with the computational approach is given in Results, where it is shown that errors introduced by this assumption are not substantial.

Since the fraction of nucleotide-free myosin head is always very small, there are only three probabilities to be computed: p_1, fraction of dissociated sites; p_2, fraction of force bonds; p_3, fraction of rigor bonds. For a potentiated unit, the steady-state values for these probabilities in a system of independent sites is derived from the three equilibrium equations obtained from the upper triangle of Fig. 1B: $\beta_1 p_1 = \beta_2 p_2$, $\beta_1 p_1 = \beta_3 p_3$, and $p_1 + p_2 + p_3 = 1$.

The solution of these equations can be written compactly in terms of [S] and three concentration-independent parameters.

$$\begin{aligned} x &= \beta_1/\beta_2;\ y = \beta_1/(\beta_3/[\mathrm{S}]);\ K = y/(1 + x); \\ p_1 &= (1 + x)^{-1}(1 + K/[\mathrm{S}])^{-1}; \\ p_2 &= (1 + x^{-1})^{-1}(1 + K/[\mathrm{S}])^{-1}; \\ p_3 &= (y/[\mathrm{S}])\, p_1. \end{aligned} \tag{2}$$

A unit loses potentiation when it makes the transition into the state (N,0,0,0), which we denote as A for convenience. The only other nonpotentiated state that need be considered (See discussion in Appendix A) is ($N - 1$,0,0,1), denoted as B. Since each dissociated myosin forms nucleotide-free myosin at the rate k_d, the rate of formation of B units is $Nk_d P(A)$, where P denotes probability. Since nucleotide-free myosin reacts with actin at the rate k_0 and with substrate at the rate k_a[S], the rate of loss of B units is $(k_0 + k_a[\mathrm{S}])\ P(B)$. Hence, the probability $P(B)$ of a unit with a nucleotide-free myosin head available for repotentiation is $Nk_d P(A)/(k_0 + k_a[\mathrm{S}])$. Since $k_d \ll$

TABLE III
RATE CONSTANTS OF THE ATPASE CYCLE

Literature estimates		Units	Base parameters
$k_a = k_5$	10^6	$M^{-1}s^{-1}$	10^6
k_d	10^{-1}	s^{-1}	2×10^{-1}
k_0	10^2	s^{-1}	10^2
k_2	10	s^{-1}	10
k_4	10 (mechanical) 100 (biochemical)	s^{-1}	3

$(k_0 + k_a[S])$ (see Table III), it follows that $P(B) \ll P(A)$ so that $P(A)$ is very nearly equal to the probability, denoted by $1 - \alpha$, that a unit is nonpotentiated. Hence, if J_+ denotes the rate of creation of potentiated units, and α the probability of a unit being potentiated, we have

$$J_+ = [Nk_d/(1 + k_a[S]/k_0)](1 - \alpha). \tag{3a}$$

Thus, as [S] increases, the rate of regeneration of potentiated units decreases, since the MgATP competes to a larger degree with actin for the nucleotide-free myosin.

Let J_- denote the rate of loss of potentiated units. Then J_- is the rate of rigor-bond breaking on units with $n_3 = 1$. The probability that a potentiated unit will have exactly one rigor bond is the product of the number of ways to select which bond is a rigor bond, N, and the probability of having $N - 1$ nonrigor bonds, $(1 - p_3)^{N-1}$. Since the transition frequency for units with one rigor bond to go to no rigor bonds is β_3,

$$J_- = (1 - p_3)^{N-1}\beta_3 N\alpha \tag{3b}$$

For a more detailed derivation of this result, see Appendix B. Equating J_+ and J_- gives the steady-state fraction of potentiated units.

$$\alpha = \frac{k_d/(1 + k_a[S]/k_0)}{\dfrac{k_d}{(1 + k_a[S]/k_0)} + \beta_3(1 - p_3)^{N-1}} \tag{4}$$

Thus when there is a large fraction of rigor bonds, α is close to unity. Another characteristic of Eq. 4 is that the larger N is, the higher [S] must be for there to be a significant fraction of nonpotentiated units. It can be seen from Eq. 3*b* that the probability of a transition from potentiated to nonpotentiated goes to zero as N increases indefinitely.

Computation of Tension, P

A readily measured mechanical property of muscle is the isometric tension. To compute this quantity it is necessary to postulate a relation between numbers of sites in each state and the mechanical properties of a muscle fiber. The following assumptions are

used: (*a*) the force exerted by the muscle is the sum of the forces exerted by each cross-bridge acting in parallel in a half-sarcomere. (*b*) At isometric conditions there is no contribution to force from rigor bonds. Assumption (*b*) is required by the very low tensions observed in the rigor state (Reuben et al., 1971; Fabiatio and Fabiato, 1975).

All results are expressed as a ratio between the computed property and its value if all bonds were in the force state. The latter quantity is indicated by a subscript 0, the bracket $< \geq$ indicates average or expected value.

$$\text{normalized force: } P/P_0 = <n_2 \quad >/N \tag{5}$$

Computations based on Eq. 1 give expected values of site states by summing over states of the cooperative units. If the states of a unit are arranged serially with index j, and the steady-state probability of a unit in state j is x_j:

$$<n_2> = \sum_j n_{2,j} x_j \tag{5a}$$

where $n_{i,j}$ denotes the value of n_i for a unit in state j.

Computations were made with the restriction that $n_4 = 0$ or 1 only, since the concentration of units with more than one nucleotide-free myosin head is very small. The number of sites in a unit was set at seven in accord with the generally accepted value (Weber and Murray, 1973).

The algebraic approximation for the expected number of sites per unit in a given state follows from the assumption of independent sites for potentiated units, with α as given by Eq. 4:

$$<n_2> = Np_2\alpha \tag{5b}$$

If there were no loss of potentiation ($\alpha = 1$) then tension would increase monotonically as [S] increased with an apparent dissociation constant of K, as can be seen from Eqs. 5*b* and 2. The maximum value of tension would be limited by the value of p_2 at very high [S], $p_2^\infty = (1 + x^{-1})^{-1}$. Thus, even without loss of potentiation, not all cross-bridges can be delivering force during an isometric tension.

RESULTS

Quantitation requires values for the rate constants of Fig. 1B. Two recent reviews of values for rate constants have been published (White and Thorson, 1973; Weber and Murray, 1973), and the values used below are taken from these sources. Since values vary between preparations as well as with salt concentration and temperature, these rate constants are order of magnitude estimates only. Furthermore, the second-order rate constants for attachment of myosin to actin were converted to first-order constants by multiplication with the myosin concentration in muscle, 10^{-4}M. The dangers of this procedure are discussed by Weber and Murray (1973). For these reasons all numbers are given to only one significant figure.

In the original model of A. F. Huxley (1957), as well as in its revised form allowing for two attached states (Julian et al., 1974), the rate constants are highly strain de-

pendent. The difference between the mechanical estimate (White and Thorson, 1973) for k_4 (10 s^{-1}) and its biochemical value (100 s^{-1}) may be due to this strain dependence. Exploratory calculations showed that a good fit to the data on the substrate dependence of tension could be obtained by very slight modification of the literature values for the rate constants (Table III): k_d was raised to 0.2 s^{-1} and k_4 was lowered to 3 s^{-1}. These parameters ("base parameters" of Table III) were used as an initial point in studies of the effect of parameter variation on the computed curves.

The substrate dependence of tension in absence of Ca is generally similar for muscle from crayfish (Reuben, et al., 1971), rabbit psoas (Zollman, et al., 1976), and frog semitendinosus and rat heart (Fabiato and Fabiato, 1975), in that the optimum pS is near 5.5 and the ratio of maximum tensions in the absence and presence of Ca are within 10% of 0.5. Fig. 2 shows that the base parameters predict an optimum at pS = 5.7 and a value of 0.47 for the ratio of maximum tension in zero-Ca to the maximum possible tension, p_2^∞.

Comparison of computations based on the exact treatment (Eq. 5*a*) to curves calculated with Eq. 5*b* is made in Fig. 2 for the base parameters of Table III. Since [S] varies over three log units, tension is plotted against pS, the negative log of [S]. A plot of p_2 *vs.* pS appears on this figure to show how tension would vary if there were

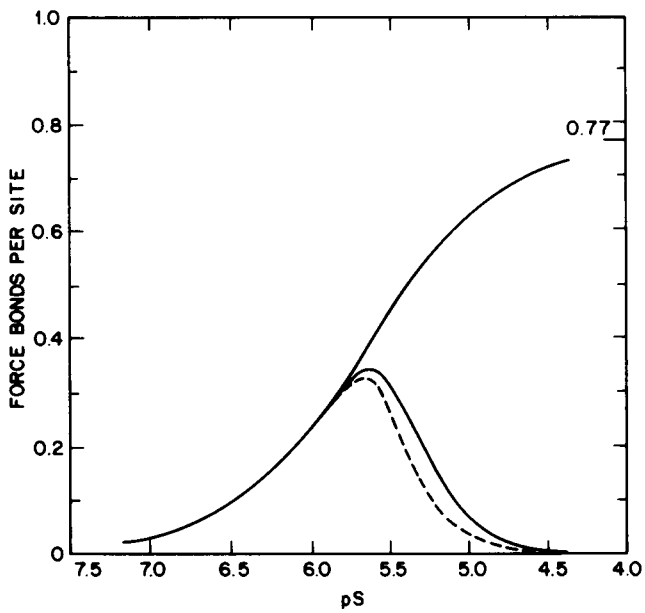

FIGURE 2 Predicted dependence of tension on substrate concentration for a muscle with the "base parameters" of Table III. The upper curve shows the variation predicted for complete potentiation, (α = 1) such as may occur with a Ca-activated muscle. In the absence of Ca the muscle would behave as shown by the full curve. The approximation of Eq. 5 *b* is shown as a dashed curve. Tension is normalized relative to the case of all bonds producing force, "force bonds/site." Concentration is represented by pS, the negative logarithm of [S]. Since α = 1 for very low [S], the three curves are initially coincident.

no loss of potentiation. The approximate curve declines slightly faster than the exact curve. Similar results were obtained over the range of parameters tested; the falling phase of the tension *vs.* pS curves was shifted at most 0.2 log units to the left for the approximate curves. Thus Eq. 5*b* represents the model well, but not perfectly, in the region of declining potentiation. Computed curves in the following figures are all based on Eq. 5*a*. Eqs. 5*b* and 4 predict that only the ratio k_a/k_0, not the individual rate constants, influences the tension. Computations based on Eq. 1 in which k_0 was varied from 30 to 1,000 s^{-1} with k_a/k_0 kept at 10^4 M^{-1} produced coincident curves of tension *vs.* pS.

It can be seen from Fig. 2 that tension is developed over the range $4 < p\text{S} < 7$. Thus variation of the ratio k_a/k_0 in the region of 10^4 M^{-1} cannot have a large effect on the substrate dependence of tension. The other parameter that enters only through the potentiation process is k_d, the rate constant for desorption of nucleotide from myosin. If one exploits the fact that $k_a[\text{S}]/k_0 \leq 1$ and approximates Eq. 4 by $\alpha = k_d/[k_d + \beta_3(1 - p_3)^{N-1}]$, it becomes clear that the rising phase of the tension *vs.* pS curve should be independent of k_d ($\alpha \sim 1$), but that as k_d increases it should require progressively higher [S], i.e., lower pS, to decrease the tension. Fig. 3 shows that as k_d varies from 0.02 to 2, the computed curves change in this manner. Since k_d increases with temperature (Malik and Martonosi, 1972; Bagshaw and Trentham, 1974), this predicts that "substrate inhibition" of tension should require higher [S] as temperature

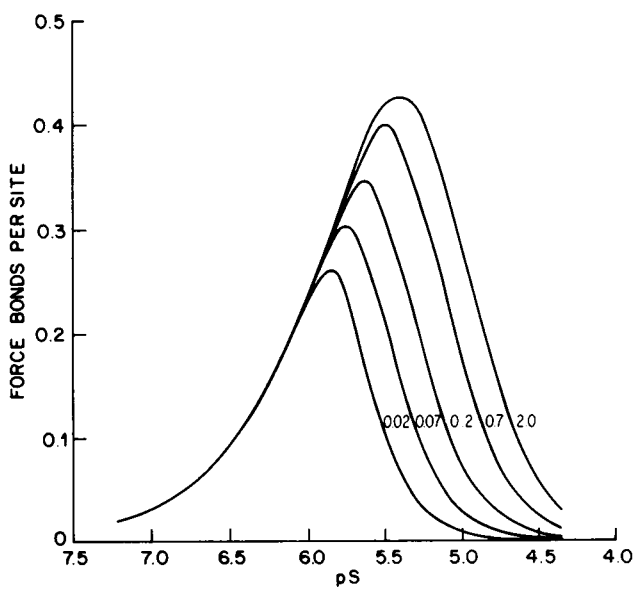

FIGURE 3 The effect of the rate constant for desorption of nucleotide from myosin, k_d, on computed curves of tension *vs* pS. Only k_d varies from its base parameter value. The value of k_d is indicated on each curve. Since k_d controls the rate of repotentiation, it influences the falling phase of the tension-pS curve.

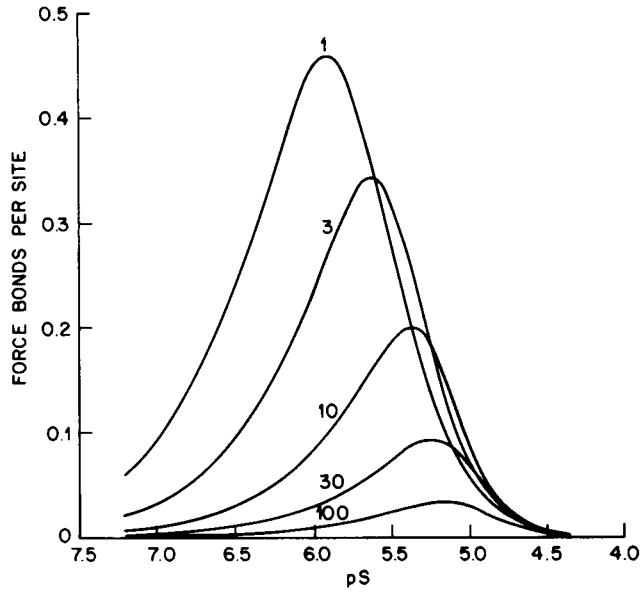

FIGURE 4 The effect of the rate constant for the transition from a force bond to a rigor bond, $k_4 = \beta_2$, on computed curves of tension *vs.* pS. Base parameter values are shown, except for k_4. Values of k_4 are indicated on each curve. Since k_4 strongly affects p_2, the average tension of potentiated units, there is a major effect on the rising side of the curves.

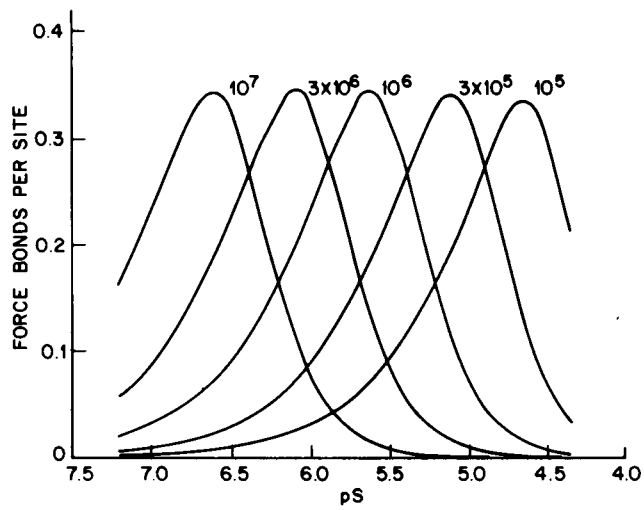

FIGURE 5 The effect of the rate constant controlling actomyosin dissociation by MgATP, $k_5 = \beta_3/[S]$, on computed curves of tension vs. pS. Base parameters were used except for k_5, the value of which is indicated on each curve. Since the only significant effect of [S] at pS > 4 is through the term β_3, a change in k_5 only shifts the curves along the pS axis.

increases. This phenomenon has been reported for skinned crayfish fibers (Brandt et al., 1972).

Variation of the kinetic parameters of the ATPase cycle also produces changes in the relation of tension to substrate concentration. From Eq. 4 it is clear that variation in β_1 and β_2 should have a minor influence on the degree of potentiation, and therefore they should not greatly influence the falling phase of the tension-substrate curve. From Eq. 2 it can be seen that the maximum attainable tension, p_2^∞, depends on $x = \beta_1/\beta_2$, and the apparent dissociation constant, K, depends on all three cycle-rate constants. The effect of varying β_2 from 3 to 100 s^{-1} is shown in Fig. 4. Note that there is a pronounced effect of β_2 on the rising phase, but rather little effect on the falling phase of the curve.

The rate constant β_3 influences both p_2 (Eq. 2) and the degree of potentiation (Eq. 4). As noted above, the approximation $\alpha = k_d/[k_d + \beta_3(1 - p_3)^{N-1}]$ is valid over a wide range in [S]. Since [S] only enters as β_3 in the probability expressions of Eq. 2, it is clear that changes in $k_5 = \beta_3/[S]$ should simply produce shifts of the tension curve along the pS axis. The simulated curves of Fig. 5 show that the model, indeed, shows the behavior.

DISCUSSION

It has been established that the approximations of Eq. 5b yield a quantitative, although imperfect, description of the model for contractile regulation. The approximate relationships are used in the following to illuminate the qualitative predictions for muscle that follow if the proposed model is correct.

Tension in the Absence of Ca

Tension is proportional to the product of two factors, p_2, the probability that a cycling bond is in the force state, and α, the probability that a cooperative unit is potentiated (Eq. 5*b*). The amplitude of tension (proportional to $(1 + \beta_2/\beta_1)^{-1}$, Eq. 2) and the position of the rising phase of the tension-pS curve (where $\alpha \sim 1$) are determined by the rate constants of the ATPase cycle. However, on the falling phase of the tension-pS curve $p_2 \sim 1$ and the curve is determined by α. Thus the left-hand cycle of Fig. 1 B, involving nucleotide-free myosin, exerts a strong effect at the superoptimal [S] by repotentiating units lost through the breaking of rigor bonds.

The relationship of [M] to the degree of potentiation, α, is shown in Fig. 6. Due to the high reactivity of nucleotide-free myosin with both actin and substrate, [M] is always very small. However, as [S] increases, [M] passes a maximum and decreases for $p\text{S} < 5.25$. Since rigor-bond breaking increases as [S] increases, and repotentiation decreases as [M] decreases; it follows that α must also fall for $p\text{S} < 5.25$. This fall in α causes the decrease in tension shown in Fig. 2. Since k_d controls the rate at which nucleotide-free myosin is formed from dissociated myosin, the falling phase of the tension-pS relation is sensitive to k_d (Fig. 3).

As indicated previously, the dramatic effect of temperature on the position of the tension-pS curve reported by Brandt et al. (1972) can be explained by the temperature

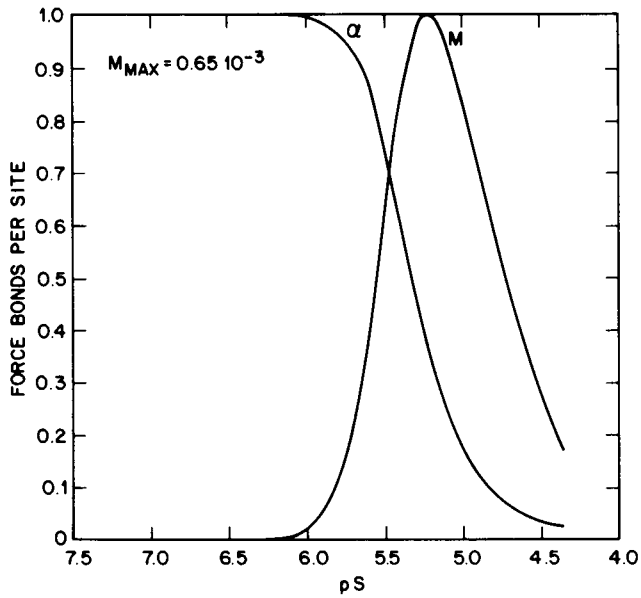

FIGURE 6 The decline of the number of nucleotide-free myosin per seven-number unit, [M], and degree of potentiation, α, as [S] increases. Note that [M] is always very small, but that [M] goes to zero as the fraction of dissociated myosin goes to zero at very low [S], as well as at high [S] where nucleotide-free myosin reacts rapidly with MgATP. At high [S], rigor bond breaking is rapid while repotentiation is slow due to the decrease in [M], causing the decline in α. Base parameters were used for all rate constants.

sensitivity of k_d, the rate constant for dissociation of nucleotide from myosin. Another phenomenon that may be explicable by this scheme is the enhancement of substrate inhibition by high concentrations of nonsubstrate forms of ATP (ATP and CaATP), reported by Zollman et al. (1976). Actomyosin does not hydrolyze these nucleotides, but they do interact strongly with myosin (Weber and Murray, 1973). These compounds may act as traps for nucleotide-free myosin, decreasing its concentration and thereby slowing repotentiation. This would cause α to decrease at lower levels of [S] as the trapping-agent concentration increased.

Effects of agents on the tension-pS curve can, in general, be interpreted in terms of changes in the rate constants, as in Results. If muscle dynamics are also measured, then it should be possible to correlate changes in the mechanical rate constants with appropriate changes in the tension-pS relation. Success of such a correlation would be an important confirmation of this model.

Ca-Induced Tension

The simplest interpretation of the action of Ca on intact myofibrils is that when it binds to troponin, a change in an associated cooperative unit occurs equivalent to potentiation. If this were true, tension would be proportional to p_2 and the tension-pS curve of a fiber saturated with Ca would be as shown in Fig. 2. At submillimolar levels of [S]

this, indeed, seems a good description both for tension (Brandt et al., 1972) and myofibrillar ATPase (Pringle, 1967; Weber, 1969). However, at higher levels of [S] there is an apparent substrate-inhibition effect even with saturating [Ca] (Zollman et al., 1976; Orentlicher, unpublished).

From data on reconstituted systems it has been proposed (Weber and Murray, 1973) that Ca binding to troponin causes an "on state" of a cooperative unit, which is less active than the "potentiated state." This would predict that at high [S] there would be a substrate-inhibition effect even at saturating [Ca], but that the tension or ATPase rate would decline to a finite level. This is in contrast to the Ca-free condition where high [S] eliminates tension. Experiments that could test this prediction with intact myofibrils have not been published.

If the Weber and Murray (1973) hypothesis is correct, then the mechanism for the transition from a nonpotentiated to a potentiated state (repotentiation) at high [S] might be different than the one we propose for the relatively low [S] of the zero-Ca tensions. At physiological [S], the rate of repotentiation through the left-hand cycle of Fig. 1 B would be slowed since a large value of k_a[S] causes a low concentration of nucleotide-free myosin, M, as shown in Fig. 6. On the other hand, the rate of formation of force bonds, AMPr, would be low but finite on nonpotentiated units in the presence of Ca (Bremel and Weber, 1972; Murray, 1975). Thus, under these conditions (high [S], high [Ca]) the repotentiation could occur via the formation of force bonds, AMPr, to produce rigor bonds, AM. Before further speculation along these lines would be profitable, experiments must demonstrate an "on state" discrete from the "potentiated" state for intact myofibrils.

Fortunately, it is unnecessary to specify a pathway for repotentiation in the presence of Ca to gain a description of the effect of Ca-binding to troponin on contractile activation. If one assumes an equilibrium between Ca-bound and Ca-free units with separate constants for potentiated and nonpotentiated units, then expressions for tension as a function of [S] and [Ca] can be derived in terms of the parameters of Eq. 2 and 4 and the two binding constants of Ca. The problem of Ca control of contractile activation will be pursued in a future paper.

The authors wish to thank Dr. M. Kawai and Dr. D. S. Wood for stimulating discussion and helpful criticism. Dr. Orentlicher was supported in part by grants to the Laboratory from National Institute of Health (HL 16082 and NS 11766), from the Muscular Dystrophy Associations of America, Inc., and from the H. Houston Merritt Clinical Research Center for Muscular Dystrophy and Related Diseases.

Received for publication 8 September 1976.

REFERENCES

ARATA, T., A. INOUE, and Y. TONOMURA. 1975. Thermodynamic and kinetic parameters of elementary steps in the reaction of H-meromyosin adenosine triphosphatase. *J. Biochem. (Tokyo).* **78**:277.

BAGSHAW, C. R., and D. R. TRENTHAM. 1974. The characterization of myosin-product complexes and of product-release steps during the magnesium-ion dependent adenosine triphosphatase reaction. *Biochemistry.* **141**:331.

BÁRÁNY, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* **50** (Suppl.):197.

BRANDT, P. W., J. P. REUBEN, and H. GRUNDFEST. 1972. Regulation of tension in the skinned crayfish muscle fiber. II. Role of calcium. *J. Gen. Physiol.* **59**:305.

BREMEL, R. D., and A. WEBER. 1972. Cooperation within actin filament in vertebrate skeletal muscle. *Nature (Lond.).* **238**:97.

BREMEL, R. D., J. M. MURRAY, and A. WEBER. 1973. Manifestations of cooperative behavior in the regulated actin filament during actin-activated ATP hydrolysis in the presence of calcium. *Cold Spring Harbor Symp. Quant. Biol.* **37**:267.

CHOCK, S. P., P. B. CHOCK, and E. EISENBERG. 1976. Pre-steady-state kinetic evidence for a cyclic interation of myosin subfragment one with actin during the hydrolysis of adenosine 5'-triphosphate. *Biochemistry.* **15**:3244.

EBASHI, S., A. KODAMA, and F. EBASHI. 1968. Troponin. I. Preparation and physiological function. *J. Biochem. (Tokyo).* **64**:465.

EISENBERG, E., and W. W. KIELLEY. 1973. Evidence for a refractory state of heavy meromyosin and subfragment-1 unable to bind to actin in the presence of ATP. *Cold Spring Harbor Symp. Quant. Biol.* **37**:145.

FABIATO, A., and F. FABIATO. 1975. Effects of magnesium on contractile activation of skinned cardiac cells. *J. Physiol. (Lond.).* **249**:497.

FILO, R. S., D. F. BOHR, and J. C. RÜEGG. 1965. Glycerinated skeletal and smooth muscle: calcium and magnesium dependence. *Science (Wash. D. C.).* **167**:1581.

HASELGROVE, J. C. 1973. X-ray evidence for a conformational change in the actin containing filaments of vertebrate striated muscle. *Cold Spring Harbor Symp. Quant. Biol.* **37**:341.

HILL, T. L., E. EISENBERG, Y. CHEN, and R. PODOLSKY. 1975. Some self-consistent two-state sliding filament models of muscle contraction. *Biophys. J.* **15**:335.

HUXLEY, A. F. 1957. Muscle structure and theories of contraction. *Prog. Biophys.* **7**:255.

HUXLEY, H. E. 1973. Structural changes in the actin- and myosin-containing filaments during contraction. *Cold Spring Habor Symp. Quant. Biol.* **37**:361.

JULIAN, F. J., K. R. SOLLINS, and M. R. SOLLINS. 1974. A model for the transient and steady-state mechanical behavior of contracting muscle. *Biophys. J.* **14**:546.

LYMN, R. W., and E. W. TAYLOR. 1970. Transient state phosphate production in the hydrolysis of nucleotide triphosphates by myosin. *Biochemistry.* **9**:2975.

LYMN, R. W., and E. W. TAYLOR. 1971. Mechanism of adenosine triphosphate hydrolysis by actomyosin. *Biochemistry.* **10**:4617.

MALIK, M. N., and A. MARTONOSI. 1972. The regulation of the rate of ATP hydrolysis by H-meromyosin. *Arch. Biochem. Biophys.* **152**:243.

MULHERN, S., E. EISENBERG, and W. W. KIELLEY. 1975. Interaction of actin with N-ethylmaleimide modified heavy meromyosin in the presence and absence of adenosine triphosphate. *Biochemistry.* **14**:3863.

MURRAY, J. 1975. Cooperative alterations in the behavior of contractile proteins. Dissertation in Biochemistry. University of Pennsylvania.

PRINGLE, J. W. S. 1967. The contractile mechanism of insect fibrillar muscle. *Prog. Biophys.* **17**:1.

REUBEN, J. P., P. W. BRANDT, M. BERMAN, and H. GRUNDFEST. 1971. Regulation of tension in the skinned crayfish muscle fiber. I. Contraction and relaxation in the absence of Ca (pCa > 9). *J. Gen. Physiol.* **57**:385.

SHIKEGAWA, M., and Y. TONOMURA. 1972. Activation of actomyosin-ATPase by tropomyosin. *J. Biochem. (Tokyo).* **71**:147.

TAYLOR, E. W. 1972. Chemistry of muscle contraction. *Annu. Rev. Biochem.* **41**:577.

TONOMURA, Y. 1972. Muscle Proteins, Muscle Contraction and Cation Transport. Tokyo University Press, Tokyo. 433 pp.

WEBER, A. 1969. Parallel response of myofibrillar contraction and relaxation to four different nucleoside triphosphates. *J. Gen. Physiol.* **53**:781.

WEBER, A., and J. M. MURRAY. 1973. Molecular control mechanisms in muscle contraction. *Physiol. Rev.* **53**:612.

WHITE, D. C. S., and J. THORSON. 1973. The kinetics of muscle contraction. *Prog. Biophys.* **27**:173.

WOOD, D. S., J. ZOLLMAN, and J. P. REUBEN. 1975. Human skeletal muscle: properties of the "chemically skinned" fiber. *Science (Wash. D. C.)* **187**:1075.

ZOLLMAN, J. R., D. S. WOOD, and J. P. REUBEN. 1976. Inhibition of Ca, substrate and rigor-tension of ATP. *Biophys. J.* **16**:124*a*. (Abstr.).

APPENDIX A

Since each site of a cooperative unit may have four states and there are N sites in a unit, the number of states of a unit would be 4^N (or 16,384 for $N = 7$) if the sites are distinguishable. However, given the number of sites, n_i, of a unit in a particular state, i, ($i = 1, 2, 3$, or 4), there is no reason to distinguish which of the N sites are in that state. Thus the cooperative unit is fully described by specifying the integer set (n_1, n_2, n_3, n_4). The number of different states of this type is equal to the number of ways of distributing N balls into four distinct cells, a standard occupancy problem in combinatories. This number is $\binom{N+3}{N} = (1/6)(N+3)(N+2)(N+1)$, which is 120 for $N = 7$. Henceforth we focus on the case $N = 7$.

At this point, the exact treatment of our model would require the solution of 120 simultaneous equations for each choice of substrate level and rate constants. However, further simplification is possible with virtually no loss of accuracy.

Since we have assumed that upon loss of potentiation, any force bond immediately breaks and becomes a dissociated (M*Pr) bond, any state having no rigor bonds ($n_3 = 0$) but having some force bonds ($n_2 > 0$) has zero probability and hence is discarded. Furthermore, as indicated in the text, nucleotide-free myosin, M, exists in extremely small concentrations compared to any other state of a cross-bridge site. Hence, we may safely neglect all states with $n_4 > 1$, that is, with more than one site with M. For nonpotentiated units the rate of repotentiation is proportional to [M] so that states with one rigor bond and one nucleotide-free myosin ($n_3 = 1$ and $n_4 = 1$) must also be considered since the loss of the rigor bond will be followed by more rapid repotentiation if M is present. However, units with nucleotide-free myosin and more than one rigor bond ($n_4 = 1$ and $n_3 > 1$) need not be considered since their concentration will be extremely small and they do not contribute to the significant pathways involving repotentiation.

Summarizing, we may eliminate all states with (*a*) $n_2 > 0$ and $n_3 = 0$, (*b*) $n_4 > 1$, and (*c*) $n_3 > 1$ and $n_4 = 1$. The remaining number of states after this simplification is 36. Computations using the base parameters and these simplifications yields 0.65×10^{-3} as the maximum probability of a unit containing M. These computations are consistent with the assumption that [M] is always very small.

The rules for identifying the allowed state transitions and associated frequencies λ_{ij} were coded into a Fortran program and a standard library subroutine was used to solve the simultaneous Eqs. 1. The solutions, normalized to $\Sigma x_j = 1$ so that the x_i represent probabilities, were used to obtain the quantities of interest. Force was calculated using Eqs. 5 and 5*a*. The probability that a unit contains nucleotide-free myosin (Fig. 6) was found by summing the probabilities for the seven states: (6, 0, 0, 1), (5, 0, 1, 1), (4, 1, 1, 1), (3, 2, 1, 1), (2, 3, 1, 1), (1, 4, 1, 1), and (0, 5, 1, 1). The probability that a unit is nonpotentiated is simply the sum of probabilities of the two states (6, 0, 0, 1) and (7, 0, 0, 0). The Fortran program is available from the authors on request.

APPENDIX B

To calculate the rate of loss of rigor bonds, we need to determine the probability of a unit having exactly one rigor bond. For brevity let R denote the event that the unit has a rigor bound at a specific site (say site 1), T denote the event that the unit has $N - 1$ nonrigor bonds at the remaining sites (sites 2, 3, . . . , N), and let Q be the event that the unit is potentiated. Then

$$P\{R \text{ and } T\} = P\{R \text{ and } T \text{ and } Q\}$$

since a unit containing a rigor bond is necessarily potentiated. Also,

$$P\{R \text{ and } T \text{ and } Q\} = P\{R|\, T \text{ and } Q\}\, P\{T|\, Q\}\, P\{Q\}$$

where the notation $P\{U \mid V\}$ denotes the conditional probability of event U given that the event V has occurred. But $P\{R|\, T \text{ and } Q\}$ is equal to one since a potentiated unit with $N - 1$ sites not in the rigor state must have a rigor bond at the remaining site. Furthermore,

$$P\{T \mid Q\} = (1 - p_3)^{N-1} \tag{B1}$$

since the $N - 1$ sites are assumed to be independent for a potentiated unit and $(1 - p_3)$ is just the probability of a site not being in the rigor state when the unit is potentiated. Also, by definition, we have $P\{Q\} = \alpha$. Hence,

$$P\{R \text{ and } T\} = \alpha(1 - p_3)^{N-1}.$$

Since there are N choices for which site has the rigor bond, the probability of a unit having one rigor bond is $NP\{R \text{ and } T\}$. Finally, since the transition rate for dissociation of a rigor bond is β_3, the rate of loss of rigor bonds, $J-$, is equal to $N\beta_3 P\{R \text{ and } T\}$, from which follows Eq. 3 *b* of the text.