COMPUTER SIMULATION OF MOVEMENT-GENERATING CROSS-BRIDGES

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ABSTRACT A stochastic computational method was developed to study properties of cross-bridge models for muscle contraction, by following the time-history of individual cross-bridges in a large population. The original two-state cross-bridge model of Andrew Huxley (1957) and a modified two-state model with more realistic behavior during steady stretching are used as examples. The method can readily compute steady-state force during shortening and stretching and force-transients following rapid changes in length. Computations of velocity with a steady load and of velocity transients are more sensitive to the randomness inherent in the stochastic method.

INTRODUCTION

This paper describes a stochastic method for computing the behavior of a population of cross-bridges of the type which have been proposed for the generation of force and movement in muscle, flagella, and other motile systems. The method follows the time-history of individual cross-bridges in a large population, to obtain the average force and cross-bridge cycle rate for the population under a variety of conditions. This method is akin to the "Monte Carlo Method" for discrete, probabilistic simulation of a continuous process, but in the present case the cross-bridge population is inherently discrete. Such methods have been used previously to analyze chemical kinetics in other contexts (cf. Chen and Hill, 1973) and their potential for dealing with complicated cross-bridge models for muscle has been pointed out by Hill (1974) and Hill et al. (1975). Computations for the cross-bridge model originally developed by Andrew Huxley (1957), which can be readily analyzed by direct calculations, are used to verify the stochastic method. Computations for a modified two-state model are presented to demonstrate the usefulness of the method for models which are more difficult to analyze by direct methods of calculation. In an accompanying paper, the method is used to analyze properties of a two-state model with oscillatory properties which might be relevant to oscillation in flagella and in insect fibrillar muscle (Brokaw, 1975). One goal for future work will be combination of the stochastic method for analyzing cross-bridge behavior with earlier methods used for simulation of the movement of flagellar models (Brokaw, 1972), in order to analyze the behavior of a flagellar model in which cross-bridges with oscillatory properties are distributed along the length of the flagellar tubules.

In these cross-bridge models, force and/or movement is generated by cross-bridges located at intervals along one type of filament (the M filament), in the course of their formation of transitory attachments to sites located at intervals along a parallel filament (the A filament). The evidence for, and the current status of, such models have been reviewed by White and Thorson (1973) and by Huxley (1974). The physical chemical principles underlying such models have been developed in a series of papers by Terrell Hill, of which Hill (1974) and Hill et al. (1975) are particularly pertinent to the present analysis. These references should be consulted for details which are not covered here.

The simplest models are "two-state" models, with one unattached state, in which a cross-bridge can exert no force on the A filament, and one attached state, in which a cross-bridge exerts a force, F, along the direction of the filaments. The force, F, is a function only of the position of the base of the cross-bridge relative to the position of its current site of attachment. These relative positions are measured by a variable, x, referred to as the "distortion" of the cross-bridge, and defined so that $F(0) = 0$. In the models considered in this paper, $F(x)$ will be a linear function containing a force constant, k_F:

$$F(x) = k_F x. \tag{1}$$

Since

$$F(-x) = -F(x), \tag{2}$$

a net force can be obtained from a population of cross-bridges only if a biased distribution of distortions of attached cross-bridges is maintained. An appropriately biased distribution can result if the cross-bridge attachment rate function, $f(x)$, and the cross-bridge detachment rate function, $g(x)$, are specified so that attachment is favored over detachment when $x > 0$, and detachment is favored over attachment when $x < 0$ (Huxley, 1957). If the resulting net force causes the A filament to slide along the M filament, cross-bridges will attach with positive distortion, do work against an external resistance to sliding as the filaments move, and then detach when the distortion falls below 0.

A free energy input is needed to operate this work-generating system by maintaining a biased distribution of attached cross-bridges. The minimal set of rate functions which can accommodate this free energy input is illustrated in Fig. 1 (Hill, 1974; Hill et al., 1975). The force exerted by an attached cross-bridge at any nonzero value of x corresponds to a quantity of free energy, $A(x)$, stored in the cross-bridge, where

$$A(x) = \int_0^x F(\hat{x})d\hat{x}. \tag{3}$$

This free energy will decrease the stability of an attached cross-bridge, as measured by a contribution of $\exp(A/kT)$ to the detachment rate functions $f'(x)$ and $g(x)$.

In order to have a substantial concentration of attached cross-bridges with positive

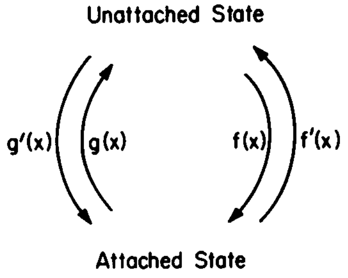

FIGURE 1 Rate functions required for a self-consistent two-state cross-bridge model (Hill, 1974; Hill et al., 1975).

distortions, $f(x)$ must be greater than $f'(x)$ in a portion of the region $x > 0$. This can result from two factors: (*a*) an inherent difference in stability between attached and unattached states, measured by the difference in their standard free energy levels at $x = 0$, and (*b*) a free energy input from a coupled chemical reaction, such as the hydrolysis of ATP. If the aggregate effect of these two factors, in units of energy$/kT$, is represented by Γ, then

$$f(x)/f'(x) = \exp[\Gamma - A(x)/kT]. \tag{4}$$

If $\Gamma > A(x)/kT$, there can be a substantial population of attached cross-bridges having a distortion x. For lower values of x, the bias towards cross-bridge attachment, described by equation 4, will increase. Therefore, in order for most cross-bridges to detach when $x < 0$, there must be another route for detachment, governed by $g(x)$ and $g'(x)$, as indicated in Fig. 1. For this reaction,

$$g'(x)/g(x) = \exp[\Gamma' - A(x)/kT], \tag{5}$$

where Γ' must depend on the difference in inherent stability of the attached and unattached states in the same way as Γ. In order to have $g'(x)/g(x)$ small near $x = 0$, Γ and Γ' must differ in their relationship to a coupled driving reaction. This is signified by

$$\Gamma - \Gamma' = \Delta, \tag{6}$$

where ΔkT is the free energy provided by the coupled chemical reaction for one cycle via the f and g transitions. ΔkT will be assumed to be the free energy provided by the hydrolysis of one molecule of ATP.

Specification of two of the quantities Γ, Γ', or Δ, and three of the functions $F(x)$, $f(x)$, $g(x)$, $f'(x)$ or $g'(x)$, is sufficient to determine the remaining two functions, using equations 4 to 6. The rate functions can then be used to set up a differential equation for the probability distribution, $n(x)$, for the distortions of attached cross-bridges in a large population of cross-bridges (cf. equation 18 of Hill et al., 1975). If this differential equation can be solved to obtain $n(x)$, the total force and cross-bridge cycle rate for the cross-bridge population can be obtained by relatively straightforward analytic or numerical integrations over the range of possible values of x. Some combinations of functions and parameters which allow the differential equation for $n(x)$ to be solved have been described by Huxley (1957), Hill et al. (1975), and by others. The following section describes a numerical procedure for obtaining force and cross-bridge cycle rate from a cross-bridge population, which does not require an explicit solution of the differential equation for $n(x)$.

COMPUTATIONAL PROCEDURE

A Fortran program was written which follows the time-history of individual cross-bridges in a large population, in order to obtain the average behavior of the cross-bridge population.

Cross-bridges are located at equal intervals, d_L, along the M filament. Each cross-bridge is indexed in sequence by an integer variable, N_L. Sites for cross-bridge attachment are located at equal intervals, d_S, along the A filament, which is parallel to the M filament. Each attachment site is indexed in sequence by an integer variable, N_S.

A variable, σ, measures shear between the filaments in terms of the current position of the origin of the attachment site sequence relative to the origin of the cross-bridge sequence. The velocity of sliding between the filaments, $V = -d\sigma/dt$, corresponds to the shortening velocity for muscle. The distance x, parallel to the filaments, between any attachment site and any cross-bridge can be obtained from:

$$x = \sigma + N_S d_S - N_L d_L. \tag{7}$$

An integer function, NSTAT, remembers the status of each cross-bridge. If the cross-bridge indexed by N_L is unattached, NSTAT $(N_L) = 0$. If the cross-bridge indexed by N_L is attached to the site indexed by N_S, then NSTAT $(N_L) = N_S$.

Specifications given for $F(x)$, $f(x)$, $g(x)$, Γ and Γ' are used to calculate $f'(x)$ and $g'(x)$ by equations 4 to 6. A table of values of the following four probability functions is then prepared for values of x in the range $-0.75 d_L \leq x \leq 0.5 d_L$, at intervals of 0.1 nm. The probability that a cross-bridge which is attached at time t is unattached at time $t + dt$ is

$$p_1(x) = [f'(x) + g(x)][1 - \exp(-Bdt)]/B, \tag{8}$$

where $B = f(x) + g(x) + f'(x) + g'(x)$. The probability that, if a cross-bridge detaches in the interval dt, it detaches via the g transition rather than via the f' transition is

$$p_2(x) = g(x)/[f'(x) + g(x)]. \tag{9}$$

The probability that a cross-bridge which is unattached at time t is attached at time $t + dt$ is

$$p_3(x) = [f(x) + g'(x)][1 - \exp(-Bdt)]/B. \tag{10}$$

The probability that, if a cross-bridge attaches in the interval dt, it attaches via the f

transition rather than via the g' transition is

$$p_4(x) = f(x)/[f(x) + g'(x)]. \quad (11)$$

The time-history of the cross-bridge population is followed by repetition of the following four steps:

Step 1 Given σ and NSTAT at time t, the status of each cross-bridge is examined. For each attached cross-bridge, N_S = NSTAT (N_L) is used to calculate x according to equation 7. The force contributed by this cross-bridge at time t is $F(x)$. The force contributions from all the attached cross-bridges are summed, and divided by N_{CB}, the total number of cross-bridges in the population, to obtain an average force per cross-bridge, P. The number of attached cross-bridges is also counted.

Step 2 A new value of σ is obtained, corresponding to the position of the filaments at $t + dt$. A predetermined protocol may be used to determine σ, or the force calculated in Step 1, and appropriate load parameters, may be used to calculate σ. For Steps 3 and 4, $\sigma = (\sigma(t) + \sigma(t + dt))/2$ is used.

Step 3 For each attached cross-bridge:

(A) x is calculated as in Step 1. The value of $p_1(x)$ for this value of x is obtained from the table. If it is larger than a random variable selected from the range 0 to 1, the cross-bridge is detached by setting NSTAT(N_L) = 0.

(B) If the cross-bridge detached in (A), and if $p_2(x)$ is less than a random variable from the range 0 to 1, an increment is subtracted from the cycle counter.

Step 4 For each unattached cross-bridge (not including those detached in Step 3):

(A) The index N_S of the site closest to $N_L d_L + x_0$ is found.

(B) The value of x corresponding to the site selected in (A) is calculated. If $p_3(x)$ is larger than a random variable from the range 0 to 1, four adjacent cross-bridges are examined. If any of them are attached to the site selected in (A), the program skips to the next unattached cross-bridge and an increment is added to the cross-bridge interference counter. If there is no interference, the cross-bridge is attached by setting NSTAT(N_L) = N_S.

(C) If the cross-bridge attached in (B), and if $p_4(x)$ is larger than a random variable from the range 0 to 1, an increment is added to the cycle counter.

The program then returns to Step 1.

The cross-bridge cycle count, after time averaging and division by N_{CB}, gives the average cross-bridge turnover rate, R, which may be interpreted as the average rate at which ATP molecules are dephosphorylated by a cross-bridge. This rate is based on the net flux $f - f'$. It could also be based on the net flux $g - g'$. These will be equivalent in steady-state situations, but can give different results in transient situations.

In Step 4, it is assumed that at any time only one site, the closest site, is potentially available for attachment of a particular cross-bridge. It is also assumed that every site is equivalent; i.e., has the same set of rate functions. There is no strong evidence that either of these simplifying conditions are met in muscle or flagella, and, in fact, some models for myosin filament structure (Squire, 1975) imply otherwise. Cross-bridge models in which these assumptions are relaxed have been discussed by Hill (1975).

In previous treatments of cross-bridge models, interference between adjacent cross-bridges has been assumed to be negligible. This assumption is not made here, because the method easily allows examination of the possibility of interference between adjacent cross-bridges. However, the examples discussed in the present paper all use a sufficiently large d_L such that no interference was observed.

COMPUTATIONS WITH HUXLEY'S 1957 MODEL

Specifications

Huxley (1957) found some simple forms for $f(x)$ and $g(x)$ which led to behavior in good agreement with measured properties of muscle. Since these functions can be readily integrated to solve the differential equation for $n(x)$, they are a convenient test of the stochastic method described in the previous section. Hill et al. (1975) have already noted the necessity to add $f'(x)$ and $g'(x)$ functions to Huxley's model in order to obtain a valid, self-consistent model, but Γ and Γ' can be chosen so that this modification has no significant impact on the results. The rate functions, and the results of analytical calculation of the force-velocity behavior, $P(V)$, and the cross-bridge cycle rate as a function of velocity, $R(V)$, are illustrated in Fig. 2. Since the

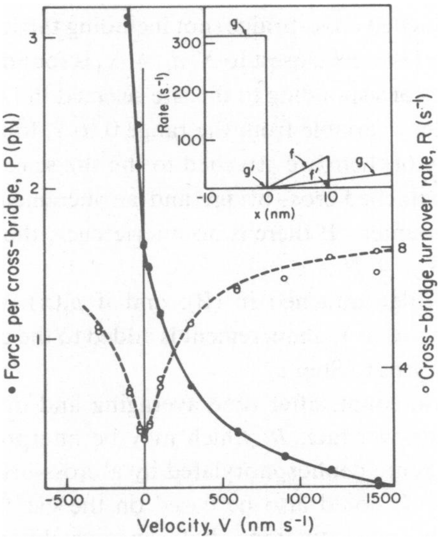

FIGURE 2 Steady-state behavior of the Huxley cross-bridge model, with rate functions illustrated in the inset. Curves are drawn from direct calculations, as described by Huxley (1957). Points are the results of stochastic computations for a population of 800 cross-bridges. Two computations, using different random number sets, were carried out for each velocity; where only one point is shown the two results were too close to resolve in the drawing.

stochastic method does not yield precise values for maximum shortening velocity, V_{max}, and isometric force, P_0, which are commonly used for normalizing parameters, it is more convenient to specify the model in terms of fully dimensioned parameters.

During isometric contraction ($V = 0$), the time average fraction of cross-bridges which are attached is

$$N_0 = \frac{f(h)}{f(h) + g(h)} (h/d_S), \tag{12}$$

where h is the largest value of x for which $f(x) > 0$, so that attachment can occur (see Fig. 2, inset). The time average force per cross-bridge at $V = 0$ is

$$P_0 = 0.5\, k_F h\, N_0. \tag{13}$$

The average cycle rate at $V = 0$ is

$$R_0 = 0.5\, g(h) N_0. \tag{14}$$

At high shortening velocities, where the probability of a cross-bridge attaching more than once during a pass past a given site is negligible, the cycle rate will be equal to the probability of attachment when passing a site multiplied by the number of sites passed per second, or

$$R_{max} = [0.5 f(h) h/V][V/d_S] = 0.5 f(h) h/d_S. \tag{15}$$

From equations 12, 14, and 15,

$$R_{max}/R_0 = \frac{f(h) + g(h)}{g(h)} \tag{16}$$

Huxley gave this ratio the value 16/3, to agree with experimental estimates based on (heat + work) measurements. Huxley also chose $g(x) = 3.919[f(h) + g(h)]$, for $x < 0$. These two assignments determine the shapes of the $P(V)$ and $R(V)$ curves, and lead to a $P(V)$ curve agreeing reasonably well with Hill's equation with $a/P_0 = 0.25$ and $(PV)_{max} = 0.1\, P_0 V_{max}$. With these assignments, $V_{max} = 2h[f(h) + g(h)]$. In order to obtain the proper relationship between isometric energy input and $P_0 V_{max}$, i.e., $P_0 V_{max} = 16\, R_0 \Delta kT$, it was necessary to specify that $0.5\, k_F h^2 = 0.75\, \Delta kT$.

The values used for h, d_S, and Δ do not influence the shapes of the $P(V)$ and $R(V)$ relationships, which are completely determined by the assignments in the preceding paragraph, but do determine the values obtained for P_0, N_0 and the relationship between V_{max} and the rate functions. Values used in the computations were $h = 10$ nm, $d_S = 37.5$ nm, and $\Delta kT = 100$ pN nm. The rate functions were specified so that $V_{max} = 1600$ nm s^{-1}, which is a representative value for frog sartorius muscle at 0° C. An appropriate value for x_0 is then $h/2 = 5$ nm. These values lead to $P_0 = 1.625$ pN, $N_0 = 0.217$, and $g(h) = 15\ s^{-1}$. A value of 42.9 nm was used for d_L. The value of d_L does not influence any of the results as long as it is large enough to avoid interference between adjacent cross-bridges and is not a multiple of d_S, so that a uniform distribution of x values is obtained in the cross-bridge population.

With the exception of $V_{\max}$, precise values for the quantities mentioned in the preceding paragraph are not known, but the values given appear to be reasonable estimates, within the limitations of our knowledge of the details of muscle filament structure (Squire, 1975). The value chosen for ΔkT, corresponding to 14.37 kcal/mol, is a numerically convenient value at the upper end of the range of physiologically reasonable values.

One additional specification, not considered by Huxley, is required (cf. Hill et al., 1975). In order to keep the reverse detachment rate, $g'(x)$, small, so that it has no influence on the behavior of the model, $\Gamma' kT$ is given a value of -17.5 pN nm, resulting in $g'(0) = 0.01\,g(0)$. The resulting value for Γ is sufficient to keep $f'(x)$ negligible—$f'(x)$ just begins to become visible in Fig. 2 (inset) as x approaches h, where $f'(h) = 8.7\ \mathrm{s}^{-1}$, and then becomes 0 when $f(x) = 0$ for $x > h$. With a smaller value of Δ, the effects of $g'(x)$ and $f'(x)$ will increase, so it is convenient to use a large value for Δ in order to compare the computed results with the results of direct calculations in which $g'(x)$ and $f'(x)$ are ignored.

Isometric Force

Computations of $P_0(t)$, for several values of cross-bridge population size, N_{CB}, and time step, dt, are illustrated in Fig. 3. In each case, the computation was begun with no cross-bridges attached, and run for the equivalent of 0.4 s. P_0 increases rapidly from 0 during the first 0.1 s, and then fluctuates around a mean value, as a result of statistical fluctuations in the number and positions of attached cross-bridges. Table I gives mean values of P_0 for these and other computations, estimated by averaging the computed values of P_0 for N_T time steps during the last half of computations such as those shown in Fig. 3. These estimates can be compared with the directly calculated value of 1.625 pN per cross-bridge.

TABLE I

INFLUENCE OF COMPUTATIONAL PARAMETERS ON MEAN ISOMETRIC FORCE

Fig. 3 label	N_T	N_{CB}	dt	P_0	ϵ
			s	*pN*	*pN*
	200	800	0.008	1.624	0.008
	200	800	0.004	1.611	0.010
	200	800	0.002	1.656	0.016
A	200	800	0.001	1.598	0.015
B	200	400	0.001	1.636	0.031
C	200	200	0.001	1.579	0.057
D	100	800	0.002	1.628	0.023
	100	800	0.001	1.604	0.017
	100	400	0.001	1.590	0.032
E	100	400	0.002	1.604	0.015
	100	400	0.004	1.639	0.033
	100	200	0.002	1.542	0.022
F	50	400	0.004	1.718	0.038
	50	200	0.004	1.466	0.016

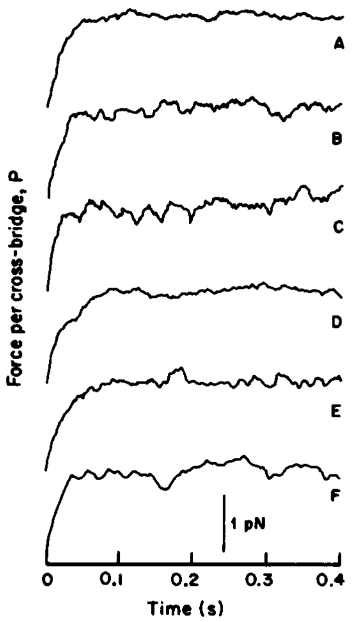

FIGURE 3

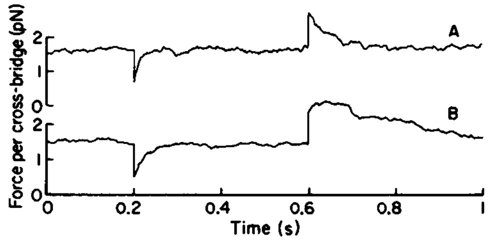

FIGURE 4

FIGURE 3 Isometric tension as a function of time for the Huxley cross-bridge model, with various computing parameters identified by the letters which refer to parameters listed in Table I.
FIGURE 4 Isometric transients computed for a population of 800 cross-bridges with $dt = 0.002$ s. In A, responses to a quick release of 3 nm and a quick stretch of 3 nm are shown for the Huxley cross-bridge model. In B, responses to a quick release of 5 nm and a quick stretch of 5 nm are shown for the modified two-state cross-bridge model.

With a population of 800 cross-bridges, statistical fluctuations in the number of attached cross-bridges would be expected to cause P_0 to vary with a standard deviation of approximately 0.06 pN. Two hundred independent measurements of P_0 would then give a mean P_0 with a standard error of approximately 0.004 pN. However, the values computed for successive time steps are not independent. The standard error, ϵ, should be estimated by formulae used for analysis of stationary time series (Bendat and Piersol, 1966):

$$N_T\epsilon^2 = C(1) + 2\sum_{m=2}^{M} C(m), \tag{17}$$

where $C(m)$ is the autocovariance function,

$$C(m) = \frac{1}{N_T - m}\sum_{n=1}^{N_T-m} [P(n) - P_0][P(n+m) - P_0]. \tag{18}$$

$P(n)$ represents the sequence of N_T values of P sampled and P_0 is the mean value. In practice, M was set at the point where $|C(m)|$ became $<0.05\,C(1)$. Computed values of ϵ are given in Table I.

On the basis of the computations summarized in Table I, values of $N_{CB} = 400$ and $N_T = 100$ have been adopted as the minimum requirement for obtaining a reasonable estimate of mean force under steady-state conditions. Larger cross-bridge populations have frequently been used for final computations, to obtain somewhat higher accuracy. Computation of ϵ has not proven to be very useful, possibly because $N_T = 100$ may not be large enough for accurate application of equations 17 and 18.

The results are not very sensitive to the choice of time step, dt, in the range of 1 to 8 ms, so that this parameter can be set as required to obtain time resolution in nonsteady-state situations.

A typical computing time for a population of 400 cross-bridges is about 0.1 s per time step on Caltech's IBM 370-158 system.

Force Transients

The computations of $P_0(t)$ can be easily modified to examine the response of the model to abrupt changes in length. Fig. 4A shows the response to a sudden 3 nm decrease in σ (quick release) and a sudden 3 nm increase in σ (quick stretch), computed with $N_{CB} = 800$ and $dt = 0.002$ s. The responses are as expected for a two-state muscle model, with a rapid drop or rise in tension resulting from the "elastic" behavior of attached cross-bridges, followed by recovery involving cross-bridge attachment and detachment to restore the population to equilibrium at the new length. The recovery following stretch appears to be about three times slower than the recovery following release. As expected, this two-state model does not show the rapid early recovery transient demonstrated experimentally by Huxley and Simmons (1971).

Force at Constant Velocity

Values of P and R at several velocities of shortening and lengthening were computed by decreasing or increasing σ by a constant increment in each time step. Results are shown by the points in Fig. 2, for comparison with the lines calculated directly. The complete set of velocities was computed twice, with different sets of random numbers. Each point represents a computation with $N_{CB} = 800$ for 200 time steps ($N_T = 100$), using $dt = 0.001$ s. Under these conditions, there is generally good agreement between the results obtained by stochastic computations and by direct calculations.

Isotonic Shortening

In the computations described so far, the force $P(t)$ has been obtained from a cross-bridge population subjected to controlled length regimes. In other important situations, $P(t)$ may be balanced by a fixed or variable load imposed on the system, and the motion $\sigma(t)$ needs to be computed. The simplest situation of this type corresponds to experiments in which the velocity of isotonic shortening of a muscle bearing a fixed load is measured. Since this is a steady-state situation, the velocity should be predictable from the $P(V)$ curve obtained at fixed velocities. However, a direct method for computing $\sigma(t)$ is needed for nonsteady situations, such as are encountered with models for flagellar oscillation.

If the applied load is a viscous resistance to shear between the filaments,

$$\sigma(t + dt) = \sigma(t) - P(t)dt/C_S, \tag{19}$$

with C_S representing the viscous resistance per cross-bridge, is a first-order approximation which can be used to obtain values of σ for Step 2 of the computation cycle. For small values of C_S, V approaches $V_{\max}$ and P approaches 0, but the fluctuations in the computed value of P do not approach 0. As a result, the fluctuations in $\sigma(t)$ become large as C_S is decreased, unless the time interval dt is also decreased. For any value of dt, there will be a lower limit to the value of C_S which can be used without the computation becoming unstable.

With a muscle (or a muscle model) it is usually assumed that the system will shorten at a velocity V which causes P to equal the applied load, and that this velocity is determined entirely by properties of the cross-bridges, with no contribution from parallel viscous resistances. In real systems, the force is balanced continuously, rather than at intervals, dt, so that no internal viscosity is needed for stability: On the other hand, since real systems are never perfectly frictionless, it may be reasonable to include some parallel viscous resistance in a model in order to obtain computational stability with finite time steps. Will inclusion of a viscosity sufficient to stabilize the computation with economically feasible time steps cause the results to be inaccurate?

A viscous resistance $C_S = C_0 \equiv 1/16{,}000$ pN nm^{-1} s per cross-bridge would add a resisting force of 0.1 pN per cross-bridge at $V = 1600$ nm s^{-1}. Addition of this C_S to the Huxley model would cause some reduction in $V_{\max}$ and power output, but would have no easily detectable effect on the shape of the $P(V)$ curve. However, this viscous resistance would give a higher, and probably noticeable, resisting force at the higher values of $V_{\max}$ which would correspond to higher temperatures. $C_S = C_0$ can be used to stabilize the computations without serious error, but it is larger than the parallel viscous resistance which might reasonably be found in a muscle at low temperature.

Fig. 5A shows the result of computing $\sigma(t)$ using equation 19, with $C_S = C_0$, $N_{CB} = 800$, and $dt = 0.001$ s. From the $P(V)$ curve in Fig. 2, the predicted V for this value of C_S is 1290 nm s^{-1}. The result in Fig. 5A has a slope corresponding to $V = 1260$ nm s^{-1}, but is barely stable. The noise in $\sigma(t)$ would cause an integration of the power output to be erroneously large, and no useful information about the isotonic transient is obtained. With $dt = 0.0025$ (Fig. 5B), the line becomes reasonably smooth, gives $V = 1250$ nm s^{-1}, and shows reasonable transient behavior. With $C_S = C_0/4$, the computation is unstable with $dt = 0.001$ s or 0.0005 s, but with $dt = 0.00025$ s it is stable, with a noisy line giving $V = 1400$ nm s^{-1}. Since computations with small dt's are expensive, some alternative methods for improving this computation have been examined.

No improvement is obtained by simply replacing $P(t)$ in equation 19 with an average of P for several time steps. The $\sigma(t)$ computation can oscillate because of the inverse relationship between P and V. A fluctuation causing a larger than average value of P will cause σ to be large, but in the subsequent time step this will cause a large reduction

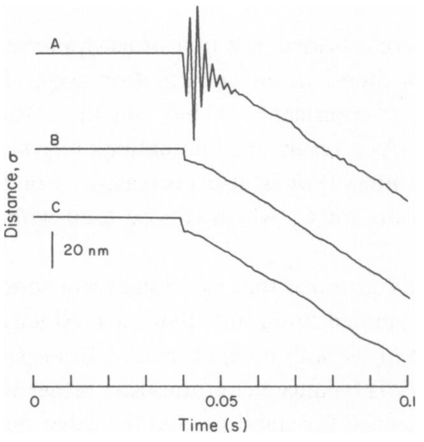

FIGURE 5

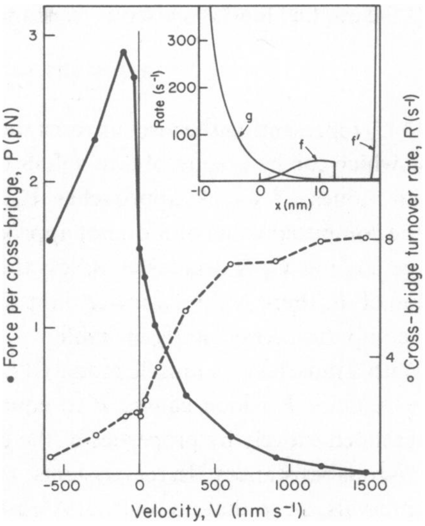

FIGURE 6

FIGURE 5 Isotonic shortening curves computed for the Huxley cross-bridge model, using a population of 800 cross-bridges, with $dt = 0.001$ s in A and C and $dt = 0.00025$ s in B. Curve C was obtained using the correction procedure discussed in the text. In each case, the cross-bridge population was allowed to approach isometric equilibrium for 0.04 s, and then released against a viscosity equivalent to 1/16,000 pN nm^{-1} s per cross-bridge.

FIGURE 6 Steady-state behavior of the modified two-state cross-bridge model, with rate functions illustrated in the inset. Computed with $N_{CB} = 800$, $N_T = 150$, and $dt = 0.002$ s.

in P, so that the following value of $d\sigma$ will be smaller than average, and so on. Averaging P simply increases the period and amplitude of these oscillations, without improving the stability. The oscillatory behavior can be reduced by anticipating the effect of $d\sigma$ on P by replacing $P(t)$ in equation 19 with $P(t) + k_F N(t) d\sigma/2$. $N(t)$ is the fraction of cross-bridges which are attached, and $k_F N(t)$ represents the stiffness of the cross-bridge population at t. The curves obtained with equation 19 modified in this manner are noticeably smoother, and stable, but noisy, results can be obtained with $C_S = C_0/4$ or $C_0/16$, with $dt = 0.001$ s. However, the velocity at $C_S = C_0$ is reduced to 1060 nm s^{-1}, so part of the improved stability may result from the decreased velocity, which could also be achieved by an increase in C_S.

This modification reduces V because it anticipates the force reduction caused by the effect of $d\sigma$ on the attached cross-bridges at t, but does not anticipate the change in force resulting from attachments and detachments during dt. During steady-state shortening, where $P(t + dt)$ should equal $P(t)$, these two effects will compensate for each other, except for the statistical fluctuations. Therefore, for steady-state shortening, the time average value of $[-k_F N(t) d\sigma/2]$ will be an estimate of the positive contributions to P resulting from cross-bridge attachments and detachments during dt. When modified to include this estimate, using an exponentially weighted running average (Rhyne, 1969) with a maximum weight of 0.1, the program gives the result shown in Fig. 5C, and a value for V of 1240 nm s^{-1}. Use of this corrective procedure does not improve the results obtained with $C_S = C_0$ at $dt = 0.00025$. However, it does provide an economical alternative to computations with very small dt, although there will be some loss of resolution of transients.

COMPUTATIONS WITH A MODIFIED TWO-STATE MODEL

A modified two-state model was examined, to demonstrate the effectiveness of the stochastic method in dealing with models in which the rate functions are chosen somewhat less arbitrarily and without regard for whether they lead to an easily integrable form of the differential equation for $n(x)$. In this model, an unattached cross-bridge is assumed to be free to rotate in the plane of the filaments, around its location on the M filament. The rate function for cross-bridge attachment, $f(x)$, is assumed to depend on the probability of finding the end of the cross-bridge at x, as a result of its rotation. This probability is governed by a Gaussian distribution function for the angular orientation of the cross-bridge.

The angular orientation of the cross-bridge is described by an angle θ, with $\theta = 0$ corresponding to the situation where a cross-bridge is perpendicular to the filaments and $x = x_0$. The Gaussian distribution is symmetrical around $\theta = 0$. For the case where the length of the cross-bridge is $x_0\sqrt{2}$,

$$\theta = \arcsin \frac{x - x_0}{x_0\sqrt{2}}, \tag{20}$$

and at $x = 0$, the neutral position of the cross-bridge, $\theta = -\pi/4$ radians.

Computations were carried out using a Gaussian function for $f(\theta)$ in the range $-\pi/4 \leq \theta \leq \pi/4$, with a variety of numerical parameters. With these $f(x)$ functions, the $f'(x)$ function becomes significant for x values somewhat greater than x_0. It was expected that the $f'(x)$ function, which allows stretched cross-bridges to detach without ATP breakdown, would give this model more realistic behavior during stretching, in contrast to the Huxley model which shows too large an increase in force and ATP turnover during stretching (Huxley, 1957). Measurements on muscle show a sharp increase in the magnitude of dP/dV as V decreases through $V = 0$, and then a drop-off of P at larger velocities of stretching (Katz, 1939; Curtin and Davies, 1975). In order to obtain reasonable behavior during stretching, it was necessary to truncate the Gaussian $f(x)$ function at $x = 1.25x_0$ and locate the sharp increase in $f'(x)$ near $x = 2x_0$ by setting $f(x) = 5 \times 10^{-10} s^{-1}$ for $x > 1.25x_0$. A $g(x)$ function, and numerical parameters, were then chosen to give $P(V)$ and $R(V)$ curves for $V > 0$ close to those obtained with the Huxley model. The results shown in Fig. 6 were obtained with

$$f(x) = 42 \exp(-4\theta^2) \ s^{-1} \qquad \text{for} \qquad 0 \leq x \leq 1.25x_0, \tag{21}$$

with $\theta(x)$ given by equation 20;

$$g(x) = 5 + 30 \exp(-0.3x) \ s^{-1}; \tag{22}$$

$x_0 = 10$ nm and $k_F = 0.9$ pN nm^{-1}. Γ, Γ', d_S, and d_L were given the same values used for computations with the Huxley model. The rate functions are illustrated in Fig. 6 (inset). In comparison to the Huxley model, this modified model has reasonable steady-state force behavior during stretching as well as during shortening, but has a slightly lower maximum work output. However, in contrast to the experimental results of Curtin and Davies (1975), the ATP turnover of this model for small velocities of stretching does not decrease to a value much less than the value at $V = 0$, and then increase for larger velocities of stretching.

Force transients for this model are shown in Fig. 4B. The recovery from a quick release is similar to, although somewhat slower than, that obtained with the Huxley model (Fig. 4A). The recovery from a quick stretch is very different, and shows a "stretch activation" followed by an extremely slow return to equilibrium. This behavior is quite unlike that observed experimentally with frog sartorius muscle, and probably results from the difference between the form of the $g(x)$ functions used in this model and in the Huxley model. In both models, the possibility for stretch-activation is present because new cross-bridge attachments can be made by cross-bridges which are suddenly brought into the region of positive $f(x)$. In the Huxley model, this effect is largely counterbalanced by the fact that all the previously attached cross-bridges experience an increased $g(x)$ following stretch, but in the modified model, the previously attached cross-bridges experience a reduced $g(x)$ following stretch. If $g(x)$ is changed to the form used for the Huxley model, the recovery following a quick stretch is faster, and no longer shows a stretch activation. However, it is still four to five times slower than the recovery following a quick release.

DISCUSSION

The stochastic method described in this paper appears to be an accurate and convenient method for evaluating the properties of cross-bridge models for force- and movement-generating systems. Once the computational program has been developed, it can be easily applied to any specification of rate functions and force function for the cross-bridges. The method will be particularly useful for analysis of more complex systems such as flagella, where the cross-bridge population is distributed along the length of the flagellar tubules and subjected to nonuniform shear patterns as a result of flagellar bending. The method may also facilitate a progression to an examination of models based on specific theoretical rationales for particular rate and force functions, in contrast to previous work where rather arbitrary functions have been chosen to give acceptable results with functions which are amenable to direct calculations. In such cases, the computational cost will probably be much less than the time cost for mathematical effort to solve the differential equation describing the cross-bridge population by other methods. However, the computing cost required to obtain accurate results is significant enough to discourage a haphazard search for models with acceptable or optimal behavior.

The computations carried out with two-state models for muscle emphasize the need to examine not only the steady-state behavior during shortening, but also the steady-state behavior during stretching and the transient behavior following length changes, in order to fully evaluate any model. The present method is easily extended to give these additional results, with negligible addition to the programming effort.

Neither of the two-state models described in this paper completely reproduces the experimentally observed behavior of muscle. It has already been suggested that two-state models are not adequate to explain other important properties of muscle (Huxley and Simmons, 1971), so that a further search for optimal two-state models may not be rewarding. The stochastic method described in this paper can form a base for extension to deal with more complicated, and hopefully more realistic, models, of the type discussed by Huxley and Simmons (1971); Julian et al. (1974); Hill (1974, 1975) and others.

My understanding of muscle models has been greatly helped by the kindness of T. L. Hill in providing me with copies of his work in advance of publication. P. Hagan has rendered valuable assistance with the computer programming.

This work has been supported by a National Institutes of Health grant (GM 21931).

Received for publication 1 March 1976.

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