# Some Mathematical Questions
# in Biology
# MUSCLE PHYSIOLOGY
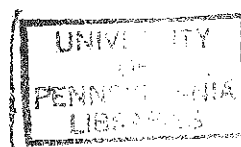
# A Mathematical Method for Unique Determination of Cross-bridge Properties from Steady-state Mechanical and Energetic Experiments on Macroscopic Muscle

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ABSTRACT. We consider cross-bridge models in which the spacing of thin filament sites is small and the cross-bridge has a strongly preferred configuration for attachment. In this case, we show that it is possible to work backwards from steady-state macroscopic experiments to uniquely determine microscopic properties of the cross-bridge. Using these properties, we solve for the transient response to step changes in load. Several features of the experimental record are predicted by the model.

1. INTRODUCTION. It is now generally accepted that striated muscle shortens when interdigitating filaments of fixed length increase their overlap by sliding past one another (see Figure 1). In 1957, H.E. Huxley and A.F. Huxley, independently, proposed a very plausible idea for the mechanism of filament sliding -- the cross-bridge hypothesis. In this paper we explore a new method for testing this idea within the context of a class

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of models similar in spirit to A.F. Huxley's 1957 scheme.
Detailed properties of the cross-bridge, previously guessed at,
will be determined uniquely by a systematic mathematical
technique that employs data from macroscopic muscle contracting
at a constant velocity. The method is tested and applied using
data of A.V. Hill (1938). The derived cross-bridge properties
are used to predict transients obtained when tetanically
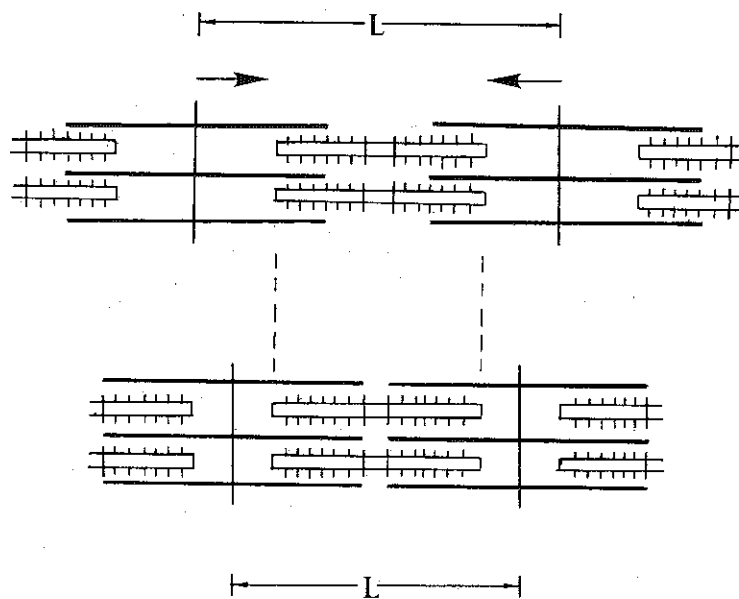stimulated muscle is subjected to sudden changes in load.



Figure 1. This figure illustrates the sliding filament mechanism
of muscle shortening. The length of the fundamental repeating
unit of striated muscle structure -- the sarcomere -- is
represented by L. The figure shows that the sarcomere contains
two types of partially overlapping filaments. During shortening,
the extent of overlap increases as the filaments slide past each
other, thereby decreasing L. The filaments themselves do not
change in length. According to the cross-bridge hypothesis,
projections from the thick filament bind during contraction to
the thin filament. These cross-bridges generate force and
through repeated cycles of attachment and detachment they produce
the relative sliding motion of the filaments.

2. BACKGROUND. Briefly, the cross-bridge theory states that
muscle force and shortening occur when molecular bridges or links
form across neighboring thick and thin filaments. The links are
formed when molecular projections located at regular intervals
along the thick filament bind to adjacent thin filaments. These
bridges form strained configurations or are placed soon after
attachment in conformations which can generate force and move
filaments past each other. In order for macroscopic shortening
to occur these molecular links must be continually breaking and
re-forming. Each cross-bridge is assumed to cycle and generate
force independently of the other cross-bridges. In 1957, A.F.
Huxley showed that these ideas were consistent with the
quantitative macroscopic behavior of muscle. More precisely, he
proposed a specific cross-bridge model that could account for the
steady-state force-velocity and energetic relationships
discovered by A.V. Hill (1938).

In order to compare the 1957 model with experiments it is
necessary to specify certain unknown cross-bridge properties.
These are the probabilities per unit time that a cross-bridge
forms or breaks in a given orientation. In addition to these
rate functions, the force a bridge produces in a given orientation
must also be specified. A.F. Huxley made reasonable guesses
about the nature of these cross-bridge rate and force functions
and adjusted these guesses by trial and error until model
predictions fit the steady-state data within experimental error.

A natural question arises when the method of trial and error
is used. Are the cross-bridge functions proposed by this
method the only ones which can satisfy the data?

This became an important physiological question when it was
subsequently discovered that the 1957 model with the specific
cross-bridge functions proposed by A.F. Huxley could not
qualitatively predict the results of certain transient
experiments performed by Civan and Podolsky (1966) and A.F.

Huxley and Simmons (1973). Podolsky and Nolan (1973), however, found a set of cross-bridge curves which could account for the Civan and Podolsky (1966) transients. Interestingly, the cross-bridge functions not only differ in shape from those originally proposed by A.F. Huxley but average values of the rate functions for bridge formation and breakage differ from those of A.F. Huxley by an order of magnitude. Like A.F. Huxley, Podolsky and Nolan employed a trial and error search until a set of cross-bridge curves were found that could satisfy their transient data. The cross-bridge curves they propose, however, cannot account qualitatively for steady-state energetics (Fenn effect) unless additional assumptions are added to the 1957 model.

3. STATEMENT OF THE PROBLEM TO BE SOLVED. Can the cross-bridge curves, described in the previous section, be obtained without guessing? We propose a systematic method which works backwards from steady-state data to determine cross-bridge functions uniquely. That is, we solve mathematically the inverse rather than the direct problem. In the direct problem, the cross-bridge functions must first be specified, then the model behavior is determined and compared to experimental behavior. In the inverse problem, experimental behavior is specified and cross-bridge functions are then derived.

4. DIFFERENCES BETWEEN THE TWO MODELS. We have solved the inverse problem for a class of models that are similar but not identical to that proposed by A.F. Huxley (1957). While it is not our aim in this paper to decide which of these two schemes is more correct, the essential differences between the two models are discussed.

We consider a class of cross-bridge models in which each cross-bridge attaches in the same configuration. The formalism

of the original 1957 scheme does not easily accommodate this idealization because receptor sites on the thin filament are assumed to be widely spaced. More precisely, the models encompassed by the 1957 scheme are single-site models—at most one thin filament site is within the attachment range of any given unattached bridge. The very tight packing of actin monomers on the thin filament has motivated T.L. Hill (1974) to consider a multisite formalism. In this paper, we idealize the multisite scheme of Hill and approximate the thin filament as a continuum of available sites. Thus, our model and A.F. Huxley's 1957 model are opposite limiting cases of a more general multisite scheme.

5. MODEL FORMULATION. As noted, we assume that every attached cross-bridge _initially_ produces force in the same configuration. If no slippage occurs during shortening then a continuum of different configurations will ensue as the attached bridge is carried along by the motion of the thin filament (see Figure 2). As the cross-bridge is carried into new configurations it produces different forces on the sliding filament. By assuming that there is a certain probability of detachment depending on configuration one can develop the following mathematical model.

Let x be the displacement (parallel to the axis of the thin filament) of an attached bridge from its starting configuration (x = 0). We define p(x), g(x) and f as follows:

p(x):   the force a bridge produces in configuration x.

g(x):   the probability per unit time (rate constant) that an attached bridge in configuration x will _detach_ from the actin filament.

f:      the rate constant for bridge attachment.

During sarcomere shortening, cross-bridges are continually

attaching, moving into new configurations, and detaching. The state of the cross-bridge population at any moment, t, will be described by a probability density function u(x,t) where $\int_a^b u(x,t)dx$ denotes the fraction of attached bridges with x in the interval [a,b] at time t. Then

$$N(t) = \int_{-\infty}^{+\infty} u(x,t)dx \leq 1 \qquad (1)$$

will define the fraction of attached bridges at t.

We now derive an equation which describes the dynamics of the cross-bridge population u(x,t).

Let v = dx/dt be the velocity of the thin filament relative to the thick filament. Note that v is positive in the direction of shortening and that the cross-bridge moves to positive values of x when the muscle is shortening. If L is the length of 1/2-sarcomere then dL/dt = -dx/dt = -v.

Consider the bridges attached in the interval [0,x]. The fraction of bridges in the interval is by definition

$$\int_0^x u(x',t)dx' \ .$$

This fraction can only change as the result of bridges moving in or out of the interval (see Figure 3). Bridges can only move into the interval by attaching in the starting configuration x = 0. This occurs at a rate proportional to the fraction of bridges available for attachment, (1 - N),

$$f(1 - N) = \text{rate of formation of attached bridges.} \qquad (2)$$

Bridges can leave the interval in one of two ways: (1) They can be carried out of the interval by the thin filament or (2) they can detach from the thin filament before they are carried away.
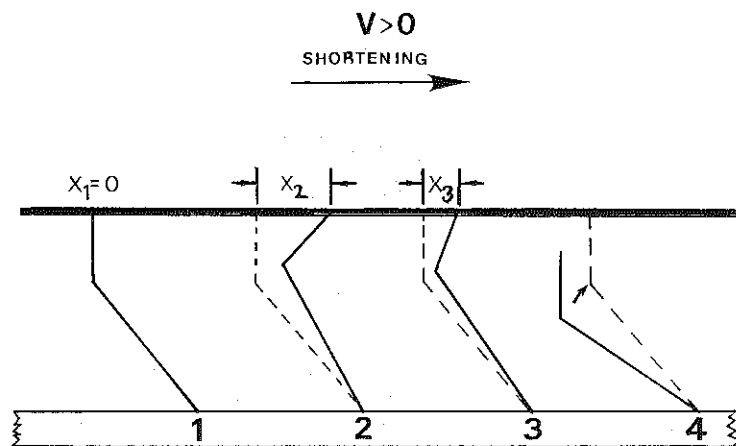
$$V > 0$$
SHORTENING



Figure 2. This diagram represents a snapshot from a left-half sarcomere. It shows the configuration of four separate cross-bridges at a given instant of time. Movement to the right (v = dx/dt > 0) represents shortening. Note that this is opposite to the usual convention in which a right-half sarcomere moves to the left during shortening. Every cross-bridge which attaches to the thin filament begins generating force in the same configuration (x = 0). Cross-bridge 1 is in this configuration. Cross-bridge 2 attached at an earlier time and has been carried along continually by the thin filament to the new configuration x = $x_2$. At any given instant every attached bridge moves along with the thin filament at the same velocity (no slipping) v = $dx_1/dt$ = $dx_2/dt$ = $dx_3/dt$ = - dL/dt. (L is the length of 1/2-sarcomere.)

Since $x_3 < x_2$, cross-bridge 2 must have attached at an earlier time than cross-bridge 3. Depending on its configuration a cross-bridge transmits a certain force, p(x), to the thin filament. In any configuration there is a certain probability per unit time, g(x), that the bridge will detach from the thin filament. Cross-bridge 4 is detached. Note that x has no meaning for a detached bridge. Re-attachment occurs at the rate f. The thin filament is idealized as a continuum of available sites for re-attachment.

Let      vu(x,t) = rate of transport out of [0,x] by
                      thin filament movement ,          (3)

$$\int_0^x g(x')u(x',t)dx' = \text{rate of detachment from the}$$
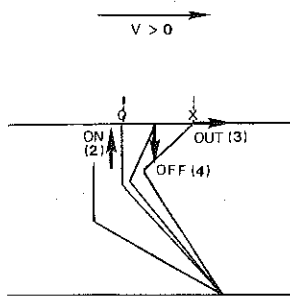$$\text{interval } [0,x] \ . \qquad (4)$$

Figure 3. The number of cross-bridges with configurations in the interval [0,x] can change by three processes that are indicated by the arrows in the figure. Cross-bridges can attach at x = 0 with the rate given by (2). Cross-bridges can be carried out of the interval [0,x] by thin filament motion. This occurs at the rate given by (3). Finally, cross-bridges in the interval can detach from the thin filament. This occurs at the rate given by (4). Considering these three processes together leads to (9) which describes the dynamics of the cross-bridge population.

Therefore, the rate at which the fraction of bridges changes in [0,x] must satisfy the following equation:

$$\frac{d}{dt}\left(\int_0^x u(x',t)dx'\right) = f\big(1 - N(t)\big) - vu(x,t) - \int_0^x g(x')u(x',t)dx' \,. \tag{5}$$

This integral equation may be used to obtain both a differential equation and the appropriate boundary condition. By setting x = 0 we obtain the following boundary condition:

$$u(0,t) = \frac{f\big(1 - N(t)\big)}{v} \,. \tag{6}$$

Bringing the time derivative in (5) inside the integral and then differentiating both sides of (5) with respect to x, we obtain

$$\frac{\partial u}{\partial t} = -v\frac{\partial u}{\partial x} - g(x)u \,. \tag{7}$$

If we consider lengthening (v < 0) instead of shortening, the boundary term becomes

$$u(0,t) = \frac{f\big(1 - N(t)\big)}{-v} \,, \tag{8}$$

but (7) remains unchanged. In summary the dynamics of the cross-bridge population with v(t) prescribed is:

$$\begin{cases} \dfrac{\partial u}{\partial t} = -v(t)\dfrac{\partial u}{\partial x} - g(x)u \,, \\[2em] u(0,t) = \dfrac{f\big(1 - N(t)\big)}{|v(t)|} \,, \\[2em] N = \displaystyle\int_{-\infty}^{+\infty} u\,dx \,. \end{cases} \tag{9}$$

In solving the inverse problem particular attention will be given to the special case v(t) ≡ v = constant.

The average force produced by a cross-bridge in the overlap-zone between thick and thin filaments (A band) is

$$P(t) = \int_{-\infty}^{+\infty} p(x)\,u(x,t)dx \,. \tag{10}$$

If ρ represents the total number of bridges available for attachment in the overlap region of 1/2-sarcomere (which is L units in length and has unit cross-sectional area), then ρN will represent the total number of attached bridges and ρP the tension produced by that 1/2-sarcomere. In general ρ will depend on L and possibly sarcoplasmic calcium concentration. In this paper we compare model behavior to experiments performed during steady tetanic contraction and over times in which the number of bridges in the overlap region is essentially constant. Thus for our purposes, ρ can be assumed constant and the integral in (10) will be proportional to the force produced by 1/2-sarcomere. If we consider a muscle composed of K

sarcomeres in series then the velocity of the muscle will be $2K \, dL/dt = -2Kv$. The force at the ends of this muscle will be $\rho P$. As usual we assume that inertial and frictional forces that arise from filament motion are negligible compared to the elastic forces generated by the cross-bridges themselves.

Before concluding this section we note that our cross-bridge equations differ from A.F. Huxley's 1957 equation in the following ways:

(1) Our cross-bridge population is described by a probability density function $u(x,t)$ with dimensions $\text{length}^{-1}$. This is different from A.F. Huxley's $n(x,t)$ which is dimensionless.

(2) The attachment term in our model is $f\big(1 - N(t)\big)$. In the 1957 scheme it is $f(x)\big(1 - n(x,t)\big)$.

Both these differences arise because we consider the actin sites to be very closely spaced and because we consider cross-bridge attachment to occur in a highly-preferred configuration.

In Huxley's model $x$ represents distance to the nearest site from the cross-bridge in question, whether the cross-bridge is attached or not. In our model, the thin filament is approximated as a continuum so the distance to the nearest site has no meaning for an unattached cross-bridge. In our model all unattached cross-bridges are equivalent.

Both models assume that cross-bridges cycle and generate force independently and that no slippage occurs while a cross-bridge is attached. This means that all attached bridges are constrained to move into new configurations at the same rate $dx/dt = dL/dt = -v$.

6. STEADY-STATE BEHAVIOR. We now consider a special case of (9) and (10) where the cross-bridge population has reached a steady-state ($\partial u/\partial t = 0$). Now $v$ and $P$ are also time

independent but related to each other. This relationship, $P(v)$, corresponds to the experimentally measured force-velocity curve. We now derive an expression for this function in the model.

For shortening ($v > 0$), the steady-state cross-bridge density can be easily shown to satisfy

$$u(x;v) = \begin{cases} \dfrac{f(1-N)}{v} \, e^{-\frac{1}{v}\int_0^x g(s)ds} & \text{for } x \geqslant 0 , \\[4mm] 0 & \text{for } x < 0 . \end{cases} \qquad (11)$$

(This solution can be checked by direct substitution into (9).)

Substituting (11) into (10) the force-velocity relationship becomes

$$P(v) = \rho \, \frac{f(1-N)}{v} \int_{-\infty}^{+\infty} p(x) \, e^{-\frac{1}{v}\int_0^x g(s)ds} \, dx . \qquad (12)$$

Equation (11) is not yet an explicit solution for $u$, since $N$ itself depends upon $u$. An explicit solution for $N$ is obtained, however, by integrating (11) from $x = -\infty$ to $x = +\infty$. This yields

$$N(v) = \frac{fI(v)}{v + fI(v)} \qquad (13)$$

where

$$I(v) = \int_0^\infty e^{-\frac{1}{v}\int_0^x g(s)ds} \, dx . \qquad (14)$$

Substituting (13) and (14) into (11) produces an explicit solution for the steady-state cross-bridge population

$$u(x;v) = \begin{cases} \dfrac{f}{v + f\int_0^\infty e^{-\frac{1}{v}\int_0^x g(s)ds} \, dx} \, e^{-\frac{1}{v}\int_0^x g(s)ds} & ; \; x \geqslant 0 , \\[6mm] 0 & ; \; x < 0 . \end{cases} \qquad (15)$$

Now $P(v)$ is obtained by substituting (15) into (10):

$$P(v) = \frac{\rho f}{v + f \int_0^\infty e^{-\frac{1}{v}\int_0^x g(s)ds} dx} \int_0^\infty p(x) e^{-\frac{1}{v}\int_0^x g(s)ds} dx \ . (16)$$

This represents a solution of the direct problem. If $f$, $g$, and $p$ are specified (for example, by guessing) then the above formula yields a definite prediction for the steady-state force-velocity curve. This, however, will not be our aim. Instead, we will use the steady-state data to derive $f$, $g$, and $p$. Before doing this we will derive some specific physiological properties of the general model in the steady-state.

### A. Maximum Shortening Velocity

Consider the case in which the sarcomere contracts against zero load. The steady velocity associated with $P = 0$ will be denoted as $v_{max}$. By (16), $v_{max}$ satisfies

$$0 = \frac{f(1 - N(v_{max}))}{v_{max}} \int_0^\infty p(x) e^{-\frac{1}{v_{max}}\int_0^x g(s)ds} dx \ . \quad (17)$$

The factor $f(1 - N)/v$ can never be zero; therefore $v_{max}$ is defined as that velocity $v$ which makes

$$\int_0^\infty p(x) e^{-\frac{1}{v}\int_0^x g(s)ds} dx = 0 \ . \quad (19)$$

Since this integral does not depend on $f$, $v_{max}$ will be unchanged by any intervention which affects the rate constant of attachment only. In general $v_{max}$ will depend on the shape of $g$ and $p$ but a simple scale change in the amplitude of $p$ will not affect $v_{max}$. On the other hand, $v_{max}$ will scale in

proportion to $g$ and this suggests one way in which muscles could be "designed" to achieve high shortening velocities.

In order for (19) to hold we must have $p(x) < 0$ for some $x$ since $e^{-(1/v)\int_0^x g(s)ds} > 0$ for all $x \geqslant 0$. If $p(0) > 0$ and $p(x)$ is continuous, then this implies that at least one equilibrium attached configuration $x_\ell$ exists such that $p(x_\ell) = 0$.

A physical interpretation of (19) is best illustrated with a simple example. Suppose $g$ is constant and $p$ is exponential as in Figure 4.

At higher velocities of shortening a greater fraction of cross-bridges cannot detach before they are carried into regions past $x_\ell$ where they exert negative force (oppose shortening). At $v_{max}$ the force produced by cross-bridges which oppose shortening is exactly balanced by the force produced by cross-bridges that are in configurations which promote shortening

$$0 = P(v_{max}) = \int_{-\infty}^{+\infty} p(x)u(x; v_{max})dx \ ,$$

$$= \int_0^{x_\ell} p(x)u(x; v_{max})dx + \int_{x_\ell}^\infty p(x)u(x; v_{max})dx \ . \quad (20)$$

Scaling up $g$ means that a bridge is less likely to be bound to the thin filament long enough to reach configurations which oppose shortening. Therefore $v_{max}$ increases.
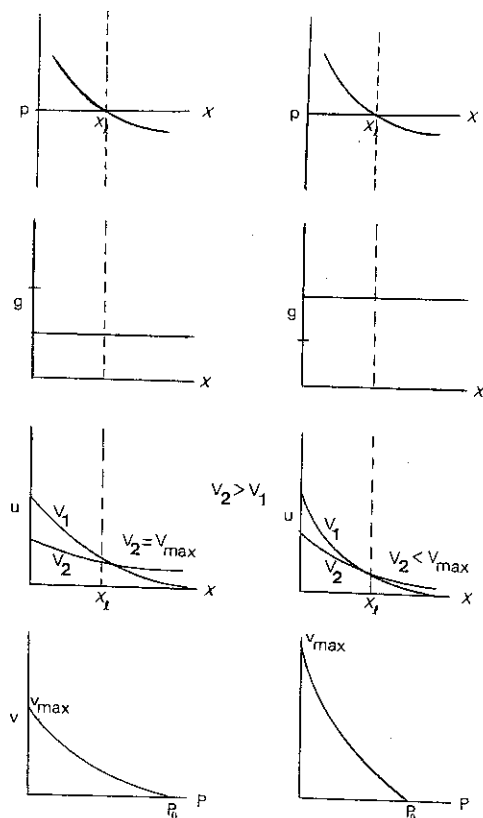
Figure 4. This figure shows the effect of scaling the amplitude of g on $v_{max}$. We consider for illustration a specific case where $g(x)$ = constant and $p(x) = \nu(e^{-\lambda(x-x_\ell)}-1)$. $x_\ell$ is the equilibrium configuration. In this case, the cross-bridge population density (Eq. (15)) is exponential $u(x;v) = [fg/(f+g)] \cdot e^{-gx/v}/v$ ; $x > 0$ and the force-velocity curve (Eq. (16)) is hyperbolic $(P+a)v = (P_0-P)b$ with $b = g/\lambda$, $P_0 = \rho p(0) f/f+g$ , and $v_{max} = p_0 b/a = g/\lambda(e^{\lambda x_\ell} - 1)$ (1/2-sarcomere). Note that the decay rate of the cross-bridge density is governed by the ratio $g/v$. Therefore configurations are less likely to exceed $x_\ell$ and oppose shortening at higher velocities if g is large. Since $v_{max}$ is the velocity where enough bridges are carried past $x_\ell$ so that their force equals the force exerted by those bridges with $x < x_\ell$, we expect that $v_{max}$ will increase when g is larger. Interestingly in this case it turns out that N, the fraction of attached bridges, is independent of v.

B.  Isometric Case (v = 0)

Consider the case in which the sarcomere is just prevented from shortening by a suitable load $P_0$. In this case all bridges bound to the thin filament will be at x = 0 and the cross-bridge density u will be a delta function. The fraction of attached bridges $N_0$ and the force $P_0$ produced in this case are:

$$N_0 = \frac{f}{f + g(0)} , \qquad P_0 = p(0)\frac{f}{f + g(0)} . \qquad (21)$$

Therefore, although f does not affect $v_{max}$ it does influence isometric tension.

If g(x) were constant, then large g would allow a muscle to achieve a high $v_{max}$ at the expense of reducing the isometric force. When we solve for g(x) using steady-state energetic data it will turn out to have a form which allows a muscle to achieve a large isometric force <u>without</u> compromising maximum shortening velocity.

7. SOLUTION OF CROSS-BRIDGE DETACHMENT FUNCTION g(x) AND FORCE FUNCTION p(x) FROM STEADY-DATA. We now consider the problem of determining the detachment function g from the energy turnover rate in the steady-state. For this purpose we focus our attention again on (14).

$$I(v) = \int_0^\infty e^{-\frac{1}{v}\int_0^x g(s)ds} dx . \qquad (14)$$

Note that if g were known, then (14) would determine I. The function g for x > 0 is transformed into the function I (v > 0) by (14). We now show that there is an inverse transformation which determines g from I. The significance of this is that I can be obtained up to a constant from steady-state energetics and therefore that g can be recovered

using such data.  The relation between  I  and steady-state  data will be discussed after it is illustrated how  g  can be recovered from  I.  Let

$$y = \int_0^x g(s)ds \quad \text{and} \quad \sigma = \frac{1}{v} \ , \tag{22}$$

then (14) can be written as

$$I\left(\frac{1}{\sigma}\right) = \int_0^\infty \left(\frac{dx}{dy}\right) e^{-\sigma y} \, dy \ . \tag{23}$$

The upper limit is  $\infty$  because we assume that  g  is bounded away from zero which is physically plausible.  Note that  $I(1/\sigma)$  is the Laplace transform of  dx/dy.  From the fundamental theorem of integral calculus  $dy/dx = g(x)$  and  therefore  $dx/dy = 1/g(x(y))$.  Define  $\bar{g}(y) \equiv g(x(y))$.  Here  $\bar{g}(y)$  may be regarded as the detachment function in a distorted distance scale.  Also  dx is related to  dy  by  $dx \cdot g(x) = dy$.  Since  $I(1/\sigma)$  is the Laplace transform of  $1/\bar{g}(y)$  we can solve for  $\bar{g}(y)$  by taking the inverse Laplace transform of  $I(1/\sigma)$

$$\bar{g}(y) = 2\pi i \left(\int_{c-i\infty}^{c+i\infty} I\left(\frac{1}{\sigma}\right) e^{\sigma y} \, d\sigma\right)^{-1} \ . \tag{24}$$

But  I  not only determines  $\bar{g}(y)$, it also allows us to recover  g(x)  by relating  x  to  y.  Since  $dx/dy = 1/\bar{g}(y)$ ,

$$x(y) = \int_0^y \frac{ds}{\bar{g}(s)} = \frac{1}{2\pi i} \int_0^y \left(\int_{c-i\infty}^{c+i\infty} I\left(\frac{1}{\sigma}\right) e^{\sigma s} \, d\sigma\right) ds \ . \tag{25}$$

This function has an inverse  y(x)  since  g > 0.  Finally  g(x)  is recovered by substituting  y(x)  into  $\bar{g}$:

$$g(x) = \bar{g}(y(x)) \ . \tag{26}$$

It remains to be shown how  I  is related to  the steady-state energy flux.

If a certain amount of chemical energy  $\varepsilon$  is released each time a cross-bridge cycles, then the energy flux  $\phi(v)$  during steady shortening is proportional to the cross-bridge turnover rate

$$\phi(v) = \rho\varepsilon f(1 - N(v)) \ . \tag{27}$$

But  N(v)  is related to  I(v)  by (13).  Substituting (13)  into (27) yields

$$I(v) = \frac{\rho\varepsilon [\phi_0 - (1-N_0)\phi]v}{\phi\phi_0} \ . \tag{28}$$

Where  $\phi_0$  and  $N_0$  are the energy turnover rate and fraction of attached bridges during steady isometric contraction.  The attachment rate  f  has been replaced by  $N_0$  using

$$\phi_0 = \rho\varepsilon f(1-N_0) \ . \tag{29}$$

The energy flux  $\phi(v)$  can be measured by determining the rate of heat and work produced during steady shortening or more directly by measuring the cross-bridge rate of ATP utilization in the steady-state.

We now apply the method used to obtain  g  in order to solve for  p(x).  From (12) the steady-state force velocity curve is

$$P(v) = \frac{\rho \ f \ (1-N(v))}{v} \int_0^\infty p(x) e^{-\frac{1}{v}\int_0^x g(s)ds} \, dx \ . \tag{12}$$

Using  $\sigma$  and  y  as defined in (22) and noting that  $\rho \frac{f(1-N(V))}{v} = \frac{\phi(v)}{\varepsilon v}$  (see (27)) we obtain

$$\frac{\varepsilon \, P(\frac{1}{\sigma})}{\sigma \phi(\frac{1}{\sigma})} = \int_0^\infty \frac{\bar{p}(y)}{\bar{g}(y)} \, e^{-\sigma y} \, dy \qquad (30)$$

where $\bar{p}(y) \equiv p(x(y))$.

The left hand side of (30) can be obtained from experiment and the right-hand side is the Laplace transform of $\bar{p}(y)/\bar{g}(y)$. We now solve for $\bar{p}(y)$

$$\bar{p}(y) = \frac{\varepsilon}{2\pi i} \, \bar{g}(y) \int_{c-i\infty}^{c+i\infty} \frac{P(\frac{1}{\sigma})}{\sigma \phi(\frac{1}{\sigma})} \, e^{\sigma y} \, d\sigma \quad . \qquad (31)$$

Finally, $p(x)$ is recovered by using (25)

$$p(x) = \bar{p}(y(x)) \, . \qquad (32)$$

The inverse problem which determines $g$, $f$, and $p$ from the steady-state data $\phi$, $N_0$, and $P$ is now solved. The flow chart in Figure 5 summarizes the method.



Figure 5. This flow-chart summarizes the steps that solve the inverse problem using steady-state data. The inputs are: (1) the force-velocity curve $P(v)$, (2) the steady-state energy flux $\phi(v)$ (heat production + power), and (3) $N_0$, the fraction of bridges attached during steady tetanic isometric contraction. The outputs are (1) the cross-bridge force function $p(x)$, (2) the cross-bridge detachment rate function $g(x)$, and (3) the rate constant, $f$, for bridge attachment. The numbers in the diagram refer to equation numbers in the text.

8. AN EXAMPLE USING THE HILL CURVES. We now test the method outlined in Figure 5 by applying it to the experimental results of A.V. Hill (1938). Although the Hill functions only approximate the behavior of muscle, their simple form make them ideal for testing the consistency of the method. The force-velocity curve is described by a hyperbola

$$P(v) = \frac{bP_0 - av}{v + b} \, , \qquad (33)$$

where $a$, $b$, and $P_0$ are positive constants that characterize a particular muscle. Note that $P_0$ is the isometric ($v = 0$) load and $v_{max} = bP_0/a$ is the shortening velocity when $P = 0$. Finally, the steady heat flux $\dot{q}$ was observed to be a linear function of the shortening velocity

$$\dot{q} = av + \phi_0 \qquad (34)$$

with $a$ as in (33)! (Later experiments (Hill, 1964) reveal a more complicated relationship.) Since the energy turnover rate, $\phi$, is the sum of the power and heat flux, we obtain

$$\phi(v) = \dot{q} + pv \equiv \phi_0 + \frac{b(a+P_0)v}{v + b} \, v = \phi_0 + (P_0-P)b \, . \qquad (35)$$

In order to compute $g$ and $p$ (see Figure 5) we must evaluate the integrals that appear in equations (24) and (31). These are

$$\frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{[\phi_0 - (1-N_0)\phi(\frac{1}{\sigma})]}{\sigma \phi(\frac{1}{\sigma})} \, e^{\sigma y} \, d\sigma \, , \qquad (36a)$$

and

$$\frac{1}{2\pi i} \int_{c-i\infty}^{c+i\infty} \frac{P(\frac{1}{\sigma})}{\sigma \phi(\frac{1}{\sigma})} \, e^{\sigma y} \, d\sigma \, . \qquad (37a)$$

If we let $v = 1/\sigma$ in (33) and (35) and substitute into (36a) and (37a) these integrals become, respectively,

$$\frac{1}{2\pi i} \oint_{\Gamma} \left[\frac{\sigma_+ + N_0(\sigma - \sigma_*)}{\sigma(\sigma - \sigma_*)}\right] e^{\sigma y} \, d\sigma , \qquad (36b)$$

and

$$\frac{1}{2\pi i} \oint_{\Gamma} \frac{(P_0 \sigma - \frac{a}{b})}{\sigma(\sigma - \sigma_*)} e^{\sigma y} \, d\sigma , \qquad (37b)$$

where $\sigma_+ = -\left(a + P_0/\phi_0\right)$ and $\sigma_* = -1/b + \sigma_+$ are both negative and $\Gamma$ is the closed path in the complex plane illustrated in Figure 6.
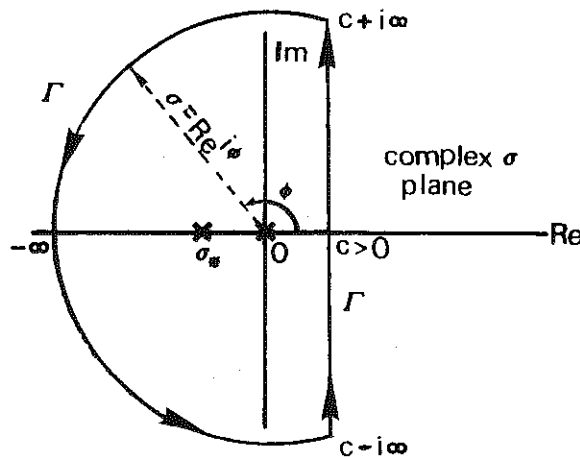


Figure 6. This figure shows the closed path $\Gamma$ in the complex plane that is used to evaluate the integrals (36b) and (37b). $\Gamma$ encloses two simple poles at $\sigma = 0$ and $\sigma = \sigma_*$. The residues at these poles are indicated in Table 1.

Note that (in each integral) $\Gamma$ encloses singularities at $\sigma = 0$ and $\sigma = \sigma_*$. The integrals are evaluated as the sum of the residues at these simple poles (see Table 1).

RESIDUES

| Pole | $\sigma = \sigma_*$ | $\sigma = 0$ |
|------|---------------------|--------------|
| Eqn. 36b | $\dfrac{\sigma_+}{\sigma_*} e^{\sigma_* y}$ | $N_0 - \dfrac{\sigma_+}{\sigma_*}$ |
| Eqn. 37b | $\left(P_0 - \dfrac{a}{b\sigma_*}\right)e^{\sigma_* y}$ | $\dfrac{a}{b\sigma_*}$ |

Table 1

The cross-bridge detachment probability function and force function in terms of $y$ are therefore

$$\bar{g}(y) = \frac{\phi_0}{\rho\varepsilon} \left[N_0 + \frac{\sigma_+}{\sigma_*} (e^{\sigma_* y} - 1)\right]^{-1} , \qquad (38a)$$

$$\bar{p}(y) = \frac{\varepsilon}{\phi_0} \bar{g}(y) \left[\frac{a}{b\sigma_*} + \left(P_0 - \frac{a}{b\sigma_*}\right) e^{\sigma_* y}\right] . \qquad (38b)$$

Since $x$ is related to $y$ by $x = \int_0^y dy'/\bar{g}(y')$ we have

$$x(y) = \frac{\rho\varepsilon}{\phi_0} \left[\left(N_0 - \frac{\sigma_+}{\sigma_*}\right)y + \frac{\sigma_+}{\sigma_*^2} (e^{\sigma_* y} - 1)\right] . \qquad (38c)$$

Equations (38) may be thought of as defining $g(x)$ and $p(x)$ because choosing a particular value of $y$, say, $y_1$, determines a value of $g$, $p$, and $x$. Namely, $g_1 = \bar{g}(y_1)$, $p_1 = \bar{p}(y_1)$, and $x_1 = x(y_1)$. Therefore $g(x_1) = g_1$ and $p(x_1) = p_1$. For example, suppose it is desired to know the detachment probability $g_0$ and force $p_0$ in the initial configuration $x = 0$. In this case $y = 0$ and substitution into (38) yields

$$g_0 = \frac{\phi_0}{\varepsilon(\rho N_0)} \,, \qquad p_0 = \frac{P_0}{\rho N_0} \,. \qquad (39)$$

The distance a cross-bridge moves from its initial configuration to its equilibrium configuration is determined by first finding $y_\ell$ such that $p(y_\ell) = 0$ and then calculating $x_\ell = x(y_\ell)$. The result is

$$y_\ell = \frac{1}{\sigma_*} \ell n \left( \frac{a}{a - P_0 b \sigma_*} \right) \,,$$

$$x_\ell = x(y_\ell) = \frac{\rho \varepsilon}{\phi_0} \left[ \left( N_0 - \frac{\sigma_+}{\sigma_*} \right) y_\ell + \frac{\sigma_+}{\sigma_*^2} \left( e^{\sigma_* y_\ell} - 1 \right) \right] \,.$$

Let us try to estimate these values for a typical muscle. For this purpose we choose

$$P_0 = 3 \text{ kgm-wt/cm}^2 \ (2.94 \times 10^6 \text{ dynes/cm}^2) \,,$$

$$\frac{a}{P_0} = 0.25 \,,$$

$$v_{max} = 1.3 \text{ muscle lengths/sec.} \,, \qquad (41)$$

$$b = \frac{a}{P_0} \cdot v_{max} = 0.325 \text{ muscle lengths/sec.} \,,$$

$$\phi_0 \simeq ab = 0.24 \text{ kgm wt muscle lengths/sec.} - cm^2$$
$\left( \text{A.V. Hill (1938)} \right)$. (This is about 27 ergs/sec. for $\frac{1}{2}$-sarcomere when it is 1.1μ long and 1 cm$^2$ in cross section.)

Using these values we obtain

$$\sigma_+ = -16.25 \left( \frac{\text{muscle lengths}}{\text{sec}} \right)^{-1} \,, \text{ and } \sigma_* = -19.95 \left( \frac{\text{muscle lengths}}{\text{sec}} \right)^{-1} \,. \qquad (42)$$

We assume that every time a cross-bridge cycles, 1 ATP molecule

is hydrolyzed yielding $\varepsilon = 7 \times 10^{-13}$ergs (10 Kcal/mole). The number of cross-bridges in the overlap zone of 1/2-sarcomere, 1.1 μ in length and 1 cm$^2$ in cross-section, has been estimated to be $6 \times 10^{12}$ (H.E. Huxley, 1960). The fraction of these which are available for attachment under conditions of steady-tetanic contraction is unknown. If we assume, for example, that this fraction is 50% and that 90% of these are attached under isometric conditions then $\rho = 3 \times 10^{12}$ and $N_0 = .90$. For this case*

$$g_0 = 14 \text{ sec}^{-1} \,,$$
$$p_0 = 10^{-6} \text{ dynes} \,,$$
$$x_\ell = 48 \text{ Å} \,,$$
$$f = 125 \text{ sec}^{-1} \,.$$

The cross-bridge cycling rate during isometric contraction is

$$f(1-N_0) = \frac{\phi_0}{\rho \varepsilon} = 12.5 \text{ per sec} \,. \qquad (44)$$

The functions $g(x)$ and $p(x)$ for this example are plotted in Figures 7(A) and 7(B). The detachment function $g(x)$ is sigmoidal with low detachment rates for configurations which generate shortening forces ($x < x_\ell$) and high detachment rates for configurations which oppose shortening ($x > x_\ell$). As mentioned in Section 6 this allows a muscle to achieve high shortening speeds without compromising its ability to lift large loads. That is, $v_{max}$ is high because $g$ is large when $x > x_\ell$ and $P_0$ is large because $g_0$ is small.

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*Instead, if we assume that $\rho = 6 \times 10^{12}$ then $x_\ell = 100$Å, $g_0 = 5 \text{ sec}^{-1}$, $f = 45 \text{ sec}^{-1}$ and the overall cycling rate is about 4.5 per sec.

Smaller values of $N_0$ increase the asymptotic values of $g$ and $p$ as $x \to \infty$,

$$g_\infty = \frac{\phi_0}{\rho\epsilon} \frac{1}{\left(N_0 - \dfrac{\sigma_+}{\sigma_*}\right)}, \qquad p_\infty = \frac{a}{\rho\sigma_* b\left(N_0 - \dfrac{\sigma_+}{\sigma_*}\right)}. \qquad (45)$$

It is important to note that even though $g$ and $p$ are finite for $x = \infty$, cross-bridges are unlikely to be found at large values of $x$ since the probability density function $u$ decays approximately like $e^{-g_\infty x/v}$ as $x$ increases past the equilibrium configuration. Note that $g_\infty \to \infty$ as $N_0 \to \sigma_+/\sigma_* = N_c$ and this occurs at a finite value of $x = x_\infty$ where

$$x_\infty \cong \frac{\rho\epsilon N_c}{P_0}, \qquad \left(\text{using } \phi_0 \cong ab, \text{ Hill (1938)}\right). \qquad (46)$$

CROSS-BRIDGE DETACHMENT CURVE

CROSS-BRIDGE FORCE VS DISTANCE
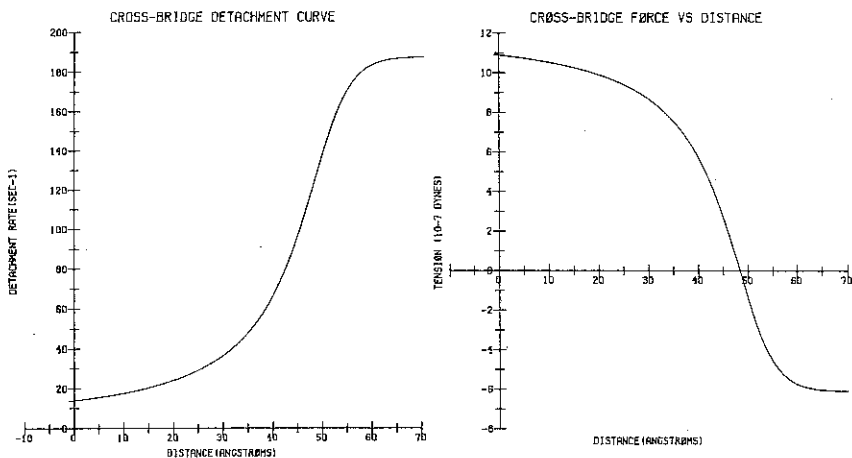
Figure 7. The cross-bridge force and detachment rate curves in these figures are obtained by solving the inverse problem using the steady-state results of A.V. Hill (1938). The Hill force-velocity and energy flux functions are the input functions in the flow-chart of Figure 5. The cross-bridge functions in this figure are output.

$g$ is no longer physical $\left(g(x) < 0 \text{ for finite } x\right)$ when $N_0 < \sigma_+/\sigma_* = N_c$. This means that $f$ must be greater than $\phi_0/\rho\epsilon(1-N_c)$ if the model is to satisfy the Hill steady-state relationships exactly for all $v \geqslant 0$. If we use parameter values previously given then

$$N_c = .81, \ x_\infty = 58\text{\AA}, \text{ and } f > 70 \text{ sec}^{-1}.$$

It can be shown that if Hill's results (33) and (34) were assumed to apply for $v < 0$ (lengthening) then (38) would also describe $g$ and $p$ for $x < 0$. Experimentally there are significant deviations from the Hill curves when $P$ exceeds $P_0$ and therefore a different relationship than (35) should be substituted to recover $g$ and $p$ for $x < 0$.

At this point we could substitute the functions $p$ and $g$ determined by the inverse method into (14) and (16) to determine $P(v)$ and

$$\phi(v) = \rho\epsilon f(1-N) = \frac{\rho\epsilon f v}{v + fI}. \qquad (47)$$

If the method works then $P(v)$ and $\phi(v)$ should, in fact, yield the Hill curves (33) and (34). Instead, we take a slightly different approach that not only tests the inverse method but also the validity of the model.

9. TRANSIENTS. Once $g$, $p$, and $f$ are specified, our model is completely determined and transient as well as steady-state behavior can be predicted. In this section we simulate transient behavior to the following experiment: muscle length is followed in time after a sudden drop in tension from steady isometric to a new constant level.

There is no reason to expect that a model with cross-bridge functions derived from steady-state data alone should correctly

predict the results of this experiment unless, of course, the model correctly embodies the essential physical mechanism that produces the transients. Therefore, strong support for the model is obtained if it can account for the qualitative features of the transients using cross-bridge functions derived from the only approximately correct steady-state curves of Hill (1938).

The transients are solved numerically by following the position of cross-bridges that attach at earlier times. Let $X_j^n$ be the position at the $n\underline{th}$ time step of those cross-bridges which attached to the thin filament at the $j\underline{th}$ time step ($j \leqslant n$) and let $U_j^n$ be the number of these bridges which are still attached at the $n\underline{th}$ time step. These numbers are updated and new cross-bridges are added during the next time step $(n+1)$ in the following order:

(1) Detachment

$$U_j^{n+1} = U_j^n - \Delta t_{n+1} \ g(X_j^n) \ U_j^{n+1} \ , \qquad j=1,\ldots,n \ , \qquad (48)$$

(2) Attachment

$$U_{n+1}^{n+1} = f\Big( 1 - \sum_{j=1}^{n} U_j^{n+1}\Big)\Delta t_{n+1} \ , \qquad (49)$$

$$X_{n+1}^{n+1} = 0 \ .$$

(3) Movement $\Delta L_{n+1}$

$$X_j^{n+1} = X_j^n + \Delta L_{n+1} \ , \qquad j=1,\ldots,n+1 \ , \qquad (50)$$

where $\Delta L_{n+1}$ is chosen to satisfy the experimental constraint of constant force production, P, given by

$$P = \sum_{j=1}^{n+1} U_j^{n+1} \ p(X_j^n + \Delta L_{n+1}) \ . \qquad (51)$$

$\Delta L_{n+1}$ is found by solving (51) using Newton's method, where

$$\Delta L_{n+1} = \sum_{k=1}^{} \Delta X_k \ ,$$

$$\Delta X_k = \frac{P - \sum_{j=1}^{n+1} U_j^{n+1} \ p\Big( X_j^{n+1} + \sum_{i=1}^{k-1} \Delta X_i\Big)}{\sum_{j=1}^{n+1} p'\Big( X_j^{n+1} + \sum_{i=1}^{k-1} \Delta X_i\Big) \ U_j^{n+1}} \ . \qquad (52)$$

The initial conditions are:

$$U_1^1 = N_0 \ ,$$

$$X_1^1 \text{ is chosen such that } U_1^1 p(X_1^1) = P \ . \qquad (53)$$

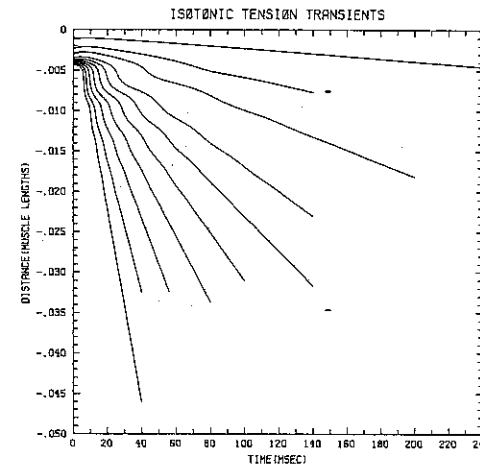That is, the method starts immediately after the jump in tension from $P_0$ to P.



Figure 8. This figure shows the predicted transient response to step changes in load when the cross-bridge functions derived from steady-state data are used (see Figure 7). At $t = 0$ the force is suddenly dropped from steady isometric to some constant force. The forces are $0.95P_0$, $0.85P_0$, $0.75P_0$, $0.6P_0$, $0.5P_0$, $0.4P_0$, $0.3P_0$, $0.2P_0$, $0.1P_0$, $0.01P_0$. The ordinate is the change in length from the initial isometric state.

Simulated transients for a series of tension steps are illustrated in Figure 8. The functions g(x) and p(x) were chosen by the inverse method described above (see Figures 7(A) and 7(B)). Three phases characterize the results: (1) a step change in length $(x_1^1)$ coincident with the step change in tension; (2) a phase in which the velocity of shortening is much less than steady-state and may even reverse $(v < 0)$; and (3) a phase in which the steady-state velocity is approached with damped oscillations. These phases also characterize the experimental findings of Huxley and Simmons (1973). The experimental transient curves are smoother than those predicted by the model. The transitions between the phases described above are less abrupt in the experimental record and oscillations are also less pronounced in comparison to the predicted transients. These discrepancies may be related to the highly idealized assumption of single point attachment or perhaps to the approximate nature of the steady-state thermal data (A.V. Hill, 1938).

Figures 9 and 10 show the velocity and energy flux $(\phi = f(1-N)\rho\epsilon)$ at the end of each curve in Figure 8. These are plotted along with A.V. Hill's curves. The nearly identical results confirm the inverse method for deriving the cross-bridge functions p and g from steady data. These results also validate the numerical method used to solve the transients.

Figure 11 shows the steady cross-bridge densities for three different loads, the densities being obtained from the end of the corresponding transients in Figure 8.
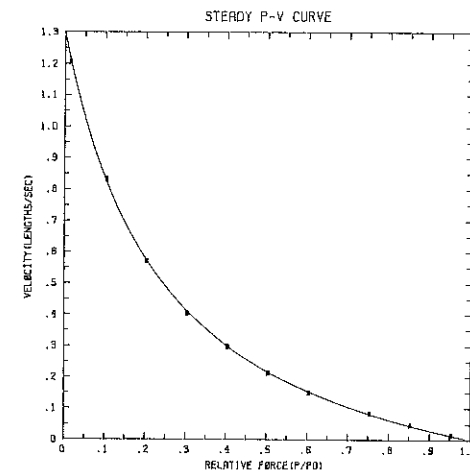
Figure 9. The solid line represents the Hill force-velocity curve (Eq. (33). $a/P_0 = 0.25$, $b = 0.325$ muscle lengths/sec). The squares represent model behavior. Each square is obtained from the end of a transient curve illustrated in Figure 8.
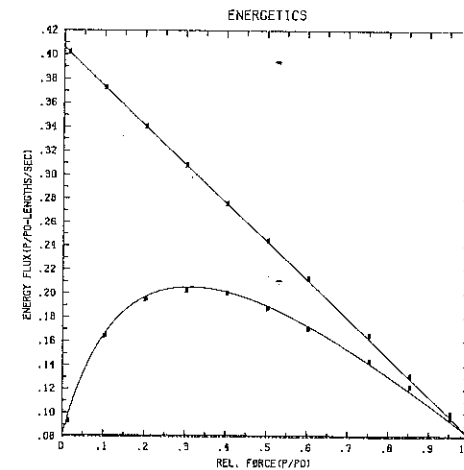


Figure 10. The solid curves represent Hill's (1938) energetic results. The squares represent model behavior. The straight line is the total energy flux (Eq. (35)). The lower curve is power + maintenance heat $(Pv + \phi_0)$. Each point representing model behavior is obtained from the end of a transient illustrated in Figure 8.
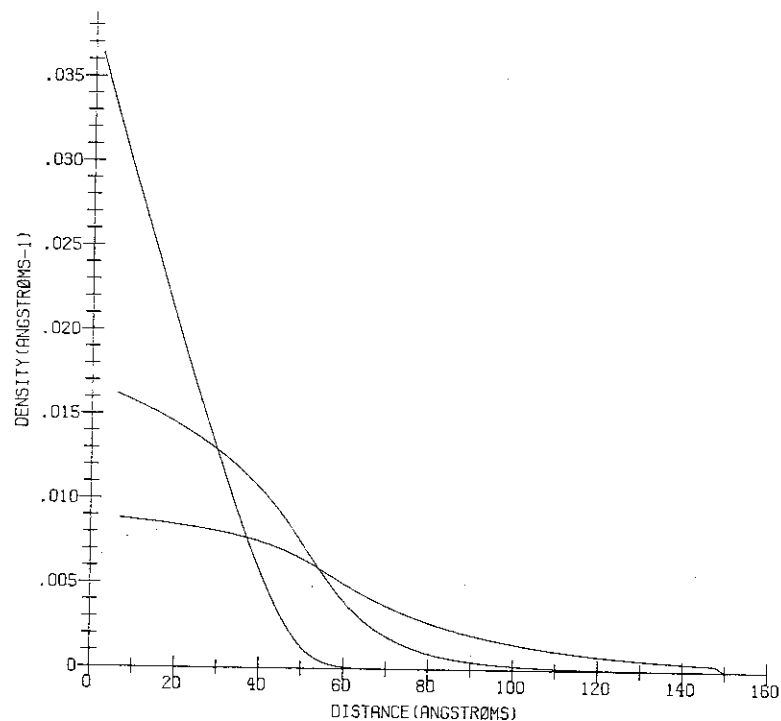
Figure 11. This figure shows the steady cross-bridge densities for three different loads: (a) $P/P_0 = 0.85$ (v = 0.05 muscle lengths/sec.), (b) $P/P_0$ 0.5 (v = 0.22 muscle lengths/sec.), and (c) $P/P_0 = 0.2$ (v = 0.57 muscle lengths/sec.). These densities were obtained from the end of the corresponding transients in Figure 8. The area under the steady-state cross-bridge density curve decreases with increasing velocity. The area in (c) is approximately 70% of the area in (a) and therefore there is a decrease in the fraction of attached bridges, N.

10. CONCLUSION. In this paper we introduce a new approach for determining cross-bridge functions. Instead of guessing at the form of the cross-bridge force and detachment rate functions, a systematic mathematical method is introduced which derives these

functions from the steady tension and energy turnover rate of muscle contracting at constant velocity.

The method is applied to a class of models similar to the one originally proposed by A.F. Huxley (1957). The principal differences between the two schemes are that we assume:

(1) sites on the thin filament for cross-bridge attachment are closely spaced;

(2) initially, cross-bridges generate force in a unique configuration.

We do not assume that thermal vibrations from an unattached equilibrium configuration are responsible for force generation. In our model, cross-bridge configurations are defined only for attached bridges. Our scheme is consistent with more detailed biochemical models in which force is generated by the release of chemical potential energy made available through ATP hydrolysis. Such detailed biochemical models are probably needed to explain the fast transients that occur during the first one or two milliseconds following sudden step changes in tension or length. We believe that the model described in this paper can only explain the slow and intermediate time-scale behavior of muscle.

The method for determining g and p is tested by using the approximate steady-velocity results of Hill (1938). In this case it is possible to solve the inverse problem exactly and determine p and g analytically.

We have used these derived cross-bridge functions in numerical simulation of model behavior to step changes in muscle load. Not only does the model satisfy the steady force-velocity and energetic curves of Hill (1938), proving that our systematic method for determining g and p is correct, but the model also predicts qualitatively the correct experimental transient behavior. Considering the accuracy of the steady-state Hill

curves, the qualitative agreement with transient behavior is very encouraging support for the model.

Our method for determining p and g can, in principle, be applied to more accurate steady-state energetic data (Hill, 1964) and force-velocity data (Edman, Mulieri, and Scubon-Mulieri, 1976) although the inversion integrals may require numerical rather than analytic methods.

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