ISOMETRIC MUSCLE CONTRACTION AND THE ACTIVE STATE: AN ANALOG COMPUTER STUDY

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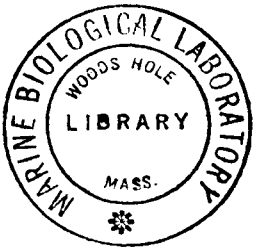
ABSTRACT From Sandow's excitation-contraction coupling hypothesis and reasonable assumptions I obtain the kinetics of the *active state*, (AS), and thence, *via* empirical equations for series elastic and contractile components for frog sartorius around 20°C, the tension, P, and dP/dt *vs.* time. Assumptions: (*a*) Rate of Ca^{+2} injection is proportional to the Ca gradient, and a permeability, which increases from zero to a limit as the membrane potential rises above a threshold. (*b*) Released Ca^{+2} is bound by the "muscle machinery," M, and removed by a carrier pump. (*c*) *The* AS *is proportional to the concentration of* Ca-M. The kinetic pattern depends mainly upon the mechanism; the time scale was fixed by the amount of Ca^{+2} injected. Depending upon the time course and repetition pattern chosen for the action potential, I obtain P and dP/dt, that agree well with experiment, for normal, potentiated, and summed twitches, tetani, and tension redevelopment after a quick release. Upon excitation the AS rises rapidly to 88%, declines thereafter in twitches, but rises slowly in unfused fashion toward 100% in tetani. The knee in dP/dt marks the first maximum in the AS. Potentiators should raise it in tetani as well as in twitches. Velocity and dP/dt show a much higher fusion frequency than P. The model integrates diverse observations. It may be tested by measuring tension and intramyofibrillar Ca^{+2} under controlled depolarization.

THEORY OF THE ACTIVE STATE

The model presented in this paper yields the time course of an isometric contraction when the time course and repetition frequency of the muscle action potential is given. It reproduces quite well the relation between the action potential and the pattern of contraction in the phenomena of potentiation, summation, and tetanus. The contraction phase is more faithfully reproduced than the relaxation. The value of a model is that it relates experimental observables in a specified manner that is susceptible of mathematical manipulation and study. Such a study will show how thoroughly the model mimics experimental reality, and will usually bring to light unforeseen relations between parameters. Thus, "model" and "theory" are not very different in meaning.



To connect the action potential with the release of activator (calcium) into sarcoplasm, I transcribed into the model Sandow's excitation-contraction coupling hypothesis (1). The amount of bound calcium is identified with the active state. Following Hill and others who employ this concept, I take the ability to bear a load, P_o, as the measure of the active state (2, 3). Finally the force of isometric contraction is made to depend on the active state, P_o, according to empirical equations, namely, Hill's force-velocity relation, and the exponential force-displacement relation of the series elastic element published by Jewell and Wilkie (4). These two relations can be combined numerically (4), or analytically (5), to yield the rate of change of tension in an isometric contraction. I have followed Sandow's suggestion of using P_o as a variable with a definite time course (5). With the facilities of an analog computer I was able to move beyond exponential kinetics, and base the time course upon a biochemically reasonable set of chemical kinetics.

THE MODEL IN DETAIL

Isometric Contraction and the Active State

The contractile property of the muscle is supposed to reside in the contractile component of length x_1, the expression of this property being Hill's force-velocity relation in the form

$$-dx_1/dt = \frac{b(P_o - P)}{P + a} \tag{1}$$

using the values $b = 4.0$ cm/sec and $a = 0.25\ P_o^{max}$ (5).

The elastic property of the muscle resides in the series elastic component of length x_2. Jewell and Wilkie's measurements (4) yield the empirical relation

$$dP/dx_2 = (P + f)/\lambda \tag{2}$$

but the values used ($f = 0.257\ P_o^{max}$ and $\lambda = 0.05$ cm) are those Sandow judged appropriate for room temperature (5). The fact that dP/dx_2 becomes a constant for $P > 0.5\ P_o^{max}$ has been ignored in this study.

Since in an isometric contraction $x_1 + x_2 =$ a constant,

$$-dx_1/dt = dx_2/dt \tag{3}$$

and since $dP/dt = (dP/dx_2)(dx_2/dt)$, we therefore obtain for the rate of development of tension in an isometric contraction

$$dP/dt = \frac{b(P + f)(P_o - P)}{\lambda(P + a)}. \tag{4}$$

This is the equation used by Sandow (5).

Sandow further suggested that P_o as representative of the active state be considered to vary with time. The question is, of course, what is an appropriate time course?

Chemical Mechanism

The following chemical mechanism, though over-simplified, is not entirely arbitrary. We may suppose that Ca released into the sarcoplasm is bound by what I purposely shall call the muscle machinery. (I have nothing to say about the nature of the binding entity.) The essential postulate for connecting equation 4 to the kinetics of Ca binding is: *the magnitude of P_o is proportional to the concentration of the complex of Ca with the muscle machinery.* For the model to encompass relaxation we can postulate a carrier pump mechanism for removing Ca from the sarcoplasm. The chemical equations are, using M for muscle machinery and Q for carrier:

$$(\mathrm{Ca}) \xrightarrow[\text{rate} = \dot{x}_{in}]{\text{action potential}} \underset{x}{\mathrm{Ca}} \tag{5}$$

$$\underset{x}{\mathrm{Ca}} + \underset{(m-\alpha)}{M} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{\alpha}{\text{Ca-}M} \tag{6}$$

$$\begin{array}{ccccc} & & \text{Ca-}\underset{\beta/2}{Q} & \longleftrightarrow & \text{Ca-}Q \\ & \overset{2k_3}{\nearrow} & & & \searrow \overset{2k_5}{} \\ \underset{x}{\mathrm{Ca}} + \underset{(q-\beta)/2}{Q} & & \text{diffusion} & & Q + \mathrm{Ca} \\ & \overset{2k_4}{\nwarrow} & & & \swarrow \end{array} \tag{7}$$

The round brackets signify those molecules and reactions that are in some other compartment than the sarcoplasm. The letters below the chemical symbols stand for the concentration of the molecules.

Equation 6 is the mechanism for the reversible binding of the activator by the muscle machinery; thus, according to postulate, the kinetics of Ca-M is the time course of the active state—that is,

$$P_o \propto \alpha \tag{8}$$

Equations 7 are the mechanism of the pump. To simplify matters, diffusion is considered to be very rapid, and then the kinetics of the pump become the same as those of the simple one-enzyme system. Equation 5 represents the injection of Ca into the myofibrillar space at a rate governed by the mechanism of excitation-contraction coupling. When a burst of Ca is released, the concentration of Ca-M rises rapidly to a near maximal value and then declines slowly, while the concentration of Ca declines steadily and almost linearly because of the action of the

pump. When the concentration of Ca approaches the value of the dissociation constant of Ca-M, the complex breaks up rapidly, and the muscle begins to relax.

From the chemical equations (5–7) the following differential equations result:

$$\dot{x} = \dot{x}_{in} - \dot{\alpha} - k_3 x(q - \beta) \tag{9}$$

$$\dot{\alpha} = k_1 x(m - \alpha) - k_2 \alpha \tag{10}$$

$$\dot{\beta} = k_3 x(q - \beta) - k_5 \beta. \tag{11}$$

The equations as written for the computer using the symbols and scaled values shown in Table I are:

$$\dot{P} = \frac{B(P + F)}{(P + A)} (P_o - P) \tag{4 C}$$

$$P_o = 10\alpha \tag{8 C}$$

$$\dot{X} = \dot{X}_{in} - \alpha - K_3 X(Q - \beta) \tag{9 C}$$

$$\dot{\alpha} = K_1 X(M - \alpha) - K_2 \alpha \tag{10 C}$$

$$\dot{\beta} = K_3 X(Q - \beta) - K_5 \beta. \tag{11 C}$$

The time dependence of the injection of Ca, viz, of $\dot{x}_{in}$ and $\dot{X}_{in}$, upon which the

TABLE I

THE STANDARD VALUES USED IN SIMULATING THE NORMAL TWITCH, BOTH IN THE MODEL AND ITS ANALOG COMPUTER REPRESENTATION

Model	Computer simulation
$P_o^{max} = 1.0$	$P_o^{max} = 100$ v*
$0 < P < 1.0$	$0 < P < 100$ v
t msec	t sec
$a = 0.25$	$A = 25$ v
$f = 0.0257$	$F = 2.57$ v
$\lambda = 0.05$ cm	$\Lambda = 0.50$
$b = 4.00$ cm/sec	$B = 0.04$
concentration of 1 μM	10 v
$m = q = 1\mu$M	$M = Q = 10$ v
x	X
$K_m = 0.28\ \mu M = k_2/k_1$	$K_M = 2.8$ v $= K_2/K_1$
$k_1 = 1.0 \times 10^9$ M^{-1} sec^{-1}	$K_1 = 0.10$ v^{-1} sec^{-1}
$k_2 = 280$ sec^{-1}	$K_2 = 0.280$ sec^{-1}
$K_q = 0.20\ \mu M = k_5/k_3$	$K_Q = 2.0$ v $= K_5/K_3$
$k_3 = 1.0 \times 10^9$ M^{-1} sec^{-1}	$K_3 = 0.10$ v^{-1} sec^{-1}
$k_4 = 0$	
$k_5 = 200$ sec^{-1}	$K_5 = 0.20$ sec^{-1}

* v = volts.

kinetic behavior of the mechanism depends, is discussed in the section on excitation-contraction coupling.

When the injection of Ca is complete in a very short time, as here, the general shape of the curve of active state vs. time depends more on the reaction mechanism than upon any particular value of the rate constants. In any case, analogy with known reactions sets some limitation upon the value one may choose for the rate constants. Table I shows the values adopted for most of this study. They were chosen with the following considerations in mind. To be sure that the kinetics of Ca-M depended strongly upon the kinetics of Ca, the rate constants k_1 and k_2 for binding Ca were made larger than the corresponding ones, k_3 and k_4, for the pump carrier. (Without loss of generality one may take $k_4 = 0$, and this was done.) I did not try the reverse dependence. It also seemed reasonable that M should be half saturated at a higher value of Ca (0.28μM) than Q is (0.20μM). Such values are compatible with experiment (6).

The maximum pumping speed was adjusted to give a duration for the active state of about 20 msec (half-life). With this value the maximum of $\dot{P}$ occurs at about 10 msec, as in frog sartorius at room temperature (7). The shape and duration of the P_o curve is similar to that presented by Jewell and Wilkie (8), after making due allowance for the difference in temperature. Moreover, it compares well with Podolsky's observation that the half time for removal of Ca in naked single fibers was about 25 msec (9).

Two parameters, k_5 and the ratio of injected Ca to q, determine the duration of the active state. The total injected Ca is $\int_0^\infty \dot{x}_{in}\, dt$ and the total amount of Ca pumped is $k_5 \int_0^\infty \beta dt$ or approximately $k_5 \beta_{max} t_{1/2,\,off}$ (10). Thus, rather more approximately,

$$t_{1/2,\,off} \cong \left(\int_0^\infty \dot{x}_{in}\, dt\right)/k_5 q. \qquad (12)$$

As Fig. 1 shows, for $q = m$, an active state curve of suitable shape and duration

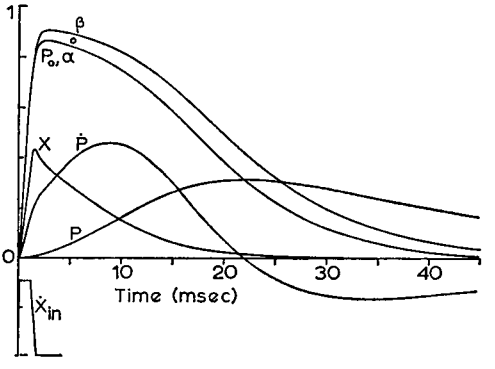


FIGURE 1 Computer solution representing a normal isometric twitch, based upon the values given in Table I. The magnitude and units of each variable is obtained by multiplying its ordinate by the factor in parentheses that follows the symbol for that variable. As appropriate, this practice is followed in the other figures. P_o (×1) active state, and also concentration of Ca-M ($\equiv \alpha$) (× 1μM); β (× 1μM) concentration of Ca-Q; P (×1) normalized isometric tension; $\dot{P}$ (× 0.05 msec^{-1}) rate of change of tension; X (× 5μM) concentration of free intramyofibrillar Ca; and $\dot{X}_{in}$ (× 10μM/msec) rate of injection of Ca.

resulted when the maximum concentration of Ca during a twitch was around $2m$, some eight times the half-saturation value for Ca binding.

Excitation-Contraction Coupling

Single stimuli Sandow's theory of excitation-contraction coupling (1) permits him to relate the potentiating effect of certain substances upon the isometric twitch to their effect upon the action potential of the muscle. The theory is that when the potential of the inside of the muscle membrane rises above a threshold —the mechanical threshold—(becoming less negative with respect to the potential of the outside) Ca is released with a flow-rate proportional to the amount by which the potential exceeds the threshold. When, however, the potential exceeds -20 mv, the Ca releasing system has achieved its maximum output and the flow remains constant. Upon repolarization, as the voltage drops below this value, the flow declines again, and ceases altogether when the potential is below the mechanical threshold. Certain potentiators are thought to raise the potential at which Ca is first released, Sandow's type *A* potentiators (1), while others clearly delay the repolarization phase of the action potential, type *B*. The gross effect of either form of potentiation is to increase the total amount of Ca released. Type *A* potentiators might be expected to begin the contraction sooner after stimulation, than in a normal twitch. Type *B* potentiators should affect contraction in the later stages. Sandow finds both these effects (1).

The profile of Ca flux vs. time, is roughly trapezoidal, and was simulated upon the computer by summing the appropriate segments of straight lines. The rising edge was given by the line,

$$V_r = -100 + 333t \ (V \text{ in volts, } t \text{ in seconds}) \tag{13}$$

the top by the flux rate limit

$$V_L = -20 \tag{14}$$

and the falling edge, for the normal action potential, by

$$V_f = 60 - 64t. \tag{15}$$

V represents the potential of the inside of the muscle with respect to the surrounding medium, volts in the computer representation standing for millivolts in real muscle.

The base of the trapezium, at the normal threshold level of -50v, is 1.57 sec equivalent to 1.57 msec in "muscle time." The actual computer equations used to

transcribe Sandow's theory are

$$\begin{aligned} \dot{x}_{\text{in}} &= 0 && \text{for} \quad V < V_{th} \\ \dot{x}_{\text{in}} &= V - V_{th} && \text{for} \quad V_{th} < V < V_L \\ \dot{x}_{\text{in}} &= V_L - V_{th} && \text{for} \quad V > V_L . \end{aligned} \tag{16}$$

For normal twitches (N), $V_{th} = -50$v, as also for B type potentiation, while for A type $V_{th} = -60$v. For B type potentiation the falling edge of the action potential is altered to

$$V_f = 60 - 46t \tag{15'}$$

For the three cases the total Ca injected is $N: A: B = 40: 56: 56$. These figures and the proportionality constant of unity in equation 16 were arrived at by trial; the final criterion being the time of the maximum in $\dot{P}$ and P. Fig. 2 shows the time dependence of equations 16.

After the model as described had been tested, and its representation of potentiation studied, the natural question to ask was whether the model could show summation and tetanus.

Repetitive stimulation The simulation of excitation-contraction coupling proved inadequate as soon as I studied the effect of repetitive stimulation. (Repetitive stimulation on the computer requires a different approach than single stimuli.) Biological experience suggests that with multiple stimuli the concentration of Ca in the sarcoplasm will rise to a steady level, with perhaps superimposed fluctuations resulting from each fresh injection of Ca. To set up a steady state there must be feedback from the sarcoplasmic Ca level to the mechanism controlling the Ca flux. Moreover, equation 16 does not represent adequately a flux controlled by a permeability barrier.

Therefore, a new representation of the excitation-contraction coupling was adopted, one that is a natural extension of Sandow's proposal; namely, the permeability π of the barrier is governed by the action potential, while the flux depends upon both this and the concentration difference between the two compart-

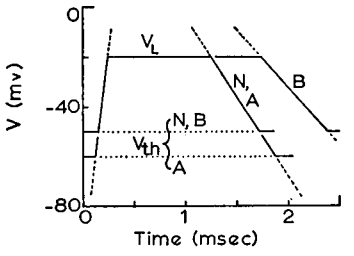


FIGURE 2 Portion of the simulated action potential relevant to excitation-contraction coupling. The solid line is the function of membrane potential used to give either $\dot{x}_{\text{in}}$ or $\pi(V)$, equation 16 or 17. N refers to a normal muscle, A and B to muscles treated with A or B type potentiators, respectively.

ments. Thus,

$$\dot{x}_{in} = \pi(V) \cdot (X_o - X) \text{ v-sec}^{-1} \tag{17}$$

The value of the average Ca concentration in the steady state depends upon the balance between outflow and inflow. Since the pump mechanism is operating at near-maximal velocity when $X = 40$v, then if in the steady state $X > 40$v, the choice of X_o and the scale of $\pi(V)$ determines the average level at a given stimulation rate. The equation finally chosen was

$$\dot{x}_{in} = \pi(V) \cdot (150 - X) \text{ v/sec} \tag{18}$$

with

$$\begin{aligned} \pi(V) &= 0 && \text{for} \quad V < V_{th} \\ \pi(V) &= (1.1/150)(V - V_{th}) \text{ sec}^{-1} && \text{for} \quad V_{th} < V < -20\text{v} \\ \pi(V) &= (1.1/150)(-20 - V_{th}) \text{ sec}^{-1} && \text{for} \quad V > -20\text{v} \end{aligned} \tag{19}$$

The main requirement was that the total amount of Ca injected with the first stimulus should be 40v as with the former representation of excitation-contraction coupling. This ensured that maximum tension in a normal twitch was the same for the two representations.

COMPUTER DETAILS

The computer equations (4 *C*, 8 *C*, 9 *C*, 10 *C*, and 11 *C*) were programmed in standard fashion on a Pace 231R analog computer (Pace, Inc., Mansfield, Ohio).[1] Equations 16 and 19 were obtained from 13, 14, and 15 by means of suitable diode limiters on integrators and adders.

For the response of the system to a single stimulus it was sufficient to start all integrators together. The action potential and resultant Ca flux is generated first and the computation of all other quantities follows from this. To obtain repetitive stimulation it is necessary to start and stop the integrators that produce the action potential separately from the rest of the computer. The relays that control those integrators were therefore fed from a panel switch. The method of operation was to start all integrators except those for the action potential in the usual fashion, and then to flip the panel switch to stimulate the system. To repeat the stimulus the panel switch was opened momentarily, resetting the integrators of that circuit to zero, and closed again to initiate another action potential. While I could have arranged to produce repeated stimuli automatically, it proved adequate to do them by hand.

[1] Courtesy of the Department of Electrical Engineering, University of British Columbia, Vancouver, Canada, to whom many thanks.

The same scheme of controlling certain integrators separately was employed to simulate quick-release experiments.

Results were recorded on both an X-Y recorder and an eight channel Brush recorder (Brush Instruments, Cleveland, Ohio).

INTERPRETATION OF THE PHASE-PLANE PLOT

A phase-plane plot permits the comparison of contractions for discerning the relative magnitude of the active state at a given value of tension.

Since

$$\dot{P} = (dP/dx_2)v \tag{20}$$

where v is the velocity of shortening of the contractile component, and dP/dx_2 is a function of P only (given by equation 2), on the phase-plane plot, vertical lines, lines of constant P, will be characterized by the same values of dP/dx_2 and of total stretch. Thus two points one above the other will differ only in the magnitude of v. Since v is held to be a function of P and the active state only, and P is the same for both, the difference between the two points lies solely in the magnitude of the active state. This theorem depends upon Hill's analysis of muscle behavior into the series elastic and contractile components, and upon the existence of a property called the active state, but not upon the specific identification of P_o as the measure of the active state, or upon Hill's equation for the force-velocity relation of the contractile component. It is the basis of several methods for determining the time course of the active state (2, 11, 12).

BEHAVIOR OF THE MODEL

The following matters were studied: (*a*) twitch shape, potentiation and the active state, and the effect of the variation of rate constants upon these; (*b*) tetani; (*c*) summation of double stimuli; (*d*) redevelopment of tension after a quick release.

Single Twitches, Potentiation and the Active State

Fig. 1 shows the isometric twitch that resulted from the values given in Table I, with adjustment of the duration of the active state to make the maximum of $\dot{P}$ and P occur at the time observed for frog sartorii at room temperature when stimulated massively (7).

Another way of assessing the fidelity of the model twitch is to present the result in a phase-plane plot, i.e. $\dot{P}$ vs. P, and compare this with experiment. Fig. 3 shows such a plot, and superimposed upon it an experimental trace from a massively stimulated frog toe muscle at room temperature.[2] The two curves are satisfyingly

[2] Florence A. Perry (Department of Pharmacology, University of British Columbia, Vancouver, Canada), and the author; unpublished experiment.

similar. That this is not the result of unequal scaling of the axes is shown by the ratio of the maximal value of the rate of increase of tension to the maximal twitch tension, $\dot{P}/_m P_m$, for the model as compared with experiment. The two differ by 4%.

Neither curve agrees in the early relaxation phase with corresponding curves published by Close (13) and Macpherson and Wilkie (14) for sartorii near 0°C.

The computed isometric myogram differs in detail from experimental curves in two points: the knee in $\dot{P}$ (first reported by Sandow (5)), is relatively higher in the experimental curve, and relaxation takes relatively longer in the published experimental curves. The knee will be discussed further in connection with the active state. The discrepancy in relaxation pattern can be seen in the phase-plane plot in Fig. 3. It can be indicated also by comparing the ratio of the time interval required for the muscle to relax from the twitch maximum to half that tension, to the interval from the stimulus to the maximum tension, viz., $t_{1/2}/t_m$. For the computed normal twitch this ratio is 1.09, while for a potentiated twitch (*A* type) it is 0.88. The corresponding values calculated from Sandow and Preiser, Fig. 1 (15) are 1.45 and 1.9. This discrepancy appears significant but has not been studied further.

Fig. 4 presents potentiated twitches of *A* and *B* type with a normal for comparison. Thus, Sandow's theory of excitation-contraction can explain potentiation. His detailed expectation, (which he verified experimentally) that $\dot{P}$ would rise sooner in the presence of an *A* type potentiator than of a *B*, or in a normal twitch, is reproduced also by the model, as shown in Fig. 5 *b*. The agreement with the results of Sandow and Preiser (15) is close.

The knee in the curve of $\dot{P}$ *vs.* time figures strongly in this comparison. What is

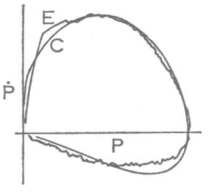


FIGURE 3 Phase-plane plots comparing a computed with an experimental normal twitch. *C*, computed *N* twitch ($\dot{P}_m/P_m$ = 0.070), and *E*, experimental record (traced) of an isometric twitch in a massively stimulated frog toe muscle at room temperature, ($\dot{P}_m/P_m$ = 0.073).

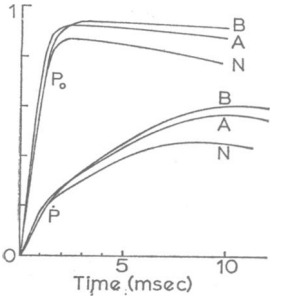


FIGURE 4 Comparison of active state and rate of change of tension curves in normal (*N*) and potentiated twitches (*A* and *B* type). (Ordinates as in Fig. 1.)

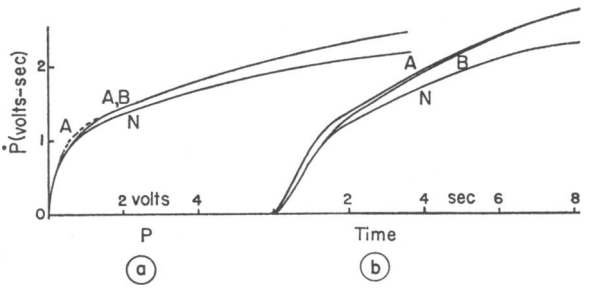


FIGURE 5 Comparison of N, A, and B type twitches. *a*. Phase-plane plot: $\dot{P}$ vs. P; *b*. $\dot{P}$ vs. time.

its origin? What is its significance? From Figs. 1 and 4 one can see that the knee coincides in time with the near arrival of P_o at its maximal value for that twitch. More than that, the knee is higher when P_o reaches a higher maximum. This is a direct result of equation 4. Equation 4 can be written

$$\dot{P} = \frac{bf}{\lambda a} P_o \frac{(1 + P/f)}{(1 + P/a)} (1 - P/P_o) \qquad (4')$$

The knee occurs when P is $\sim$1.5v (Fig. 5), comparable to f, but much less than a or P_o. Thus, to within 10%

$$\dot{P} = \frac{bf}{\lambda a} P_o(1 + P/f). \qquad (21)$$

To the degree that b, f, λ, and a are good constants independent of the state of activity of the muscle, the time course of $\dot{P}$, up to slightly beyond the knee, reflects the kinetics of the active state.

The early part of the phase-plane plot of $\dot{P}$ *vs.* P (Fig. 5 *a*) also shows the knee. In this plot the difference between N, A, and B twitches for a given P depend only upon the magnitude of the active state. Thus, at low P, early in the contraction, the active state in A exceeds that of N or B, while at higher P the active states of A and B are the same but greater than in N. The conclusion, therefore, that the knee reflects the magnitude of the active state does not depend necessarily upon the assumption that P_o is the correct measure of it. If, as I expect, experimental phase plane plots are quite similar to Fig. 5 *a*, then the conclusion may be drawn independently of this model. The conclusion will not, however, be independent of Hill's analysis, since the concept of active state is part of that analysis (11), and the interpretation of the phase-plane plot depends on it.

The prominence of the knee depends to some extent upon the speed with which P_o approaches its maximum. This is apparent in Fig. 6, which shows how the kinetics of the bound Ca-M depend upon the rate constants for the binding reaction.

What governs the knee height relative to the maximum of $\dot{P}$ is not clear. Whether increasing $bf/\lambda a$ (see equation 20) would improve agreement, without altering the kinetics of the active state, cannot be answered without further study on a computer.

Fig. 6 demonstrates the point made earlier that the general agreement of the computer solution with experiment does not depend upon a very special choice of rate constants. Fig. 7 shows that speeding up the pump, by shortening the active state, alters the myogram, but that changing the affinity of the pump, K_q, without changing the rate very much, has correspondingly less effect on the myogram. As expected, the general shape of the active state curve, its early maximum, slow decline (rather than "plateau"), and its final rapid decay is the result of the binding equilibrium and the more or less linear decay of the ligand concentration caused by the pump.

Single twitches are unchanged when the extended theory of excitation-contraction coupling was used (equation 19).

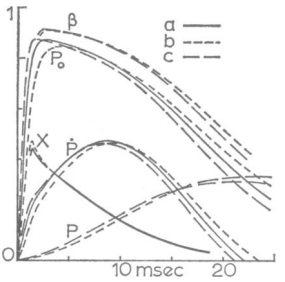


FIGURE 6 Dependence of the computer solution upon the rate constants for the binding of Ca by muscle machinery. The binding constant is nearly the same for each curve. *a*. $k_1 = 1.0 \times 10^9$ M^{-1} sec^{-1}, $k_2 = 280$ sec^{-1}, the *N* twitch. *b*. $k_1 = 0.50 \times 10^9$ M^{-1} sec^{-1}, $k_2 = 150$ sec^{-1}. *c*. $k_1 = 2.0 \times 10^9$ M^{-1} sec^{-1}, $k_2 = 600$ sec^{-1}. Where for clarity the *a* curve is omitted, it lies between the others. For other constants see Table I. (Ordinates as in Fig. 1.)

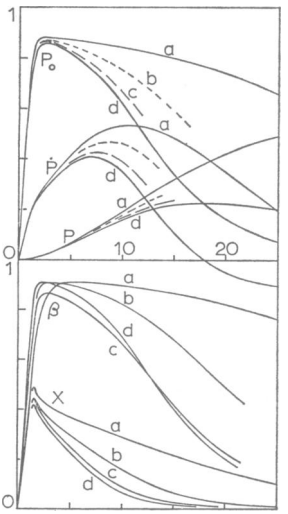


FIGURE 7 The effect upon the computer solution of varying the pump rate constants and affinity. *a*. $k_3 = 0.5 \times 10^9$ M^{-1} sec^{-1}, $k_5 = 100$ sec^{-1} and so $K_q = 0.20$ μM. *b*. $k_3 = 1.0 \times 10^9$ M^{-1} sec^{-1}, $k_5 = 200$ sec^{-1}, and $K_q = 0.20$ μM, the *N* twitch, *c*. $k_3 = 1.0 \times 10^9$ M^{-1} sec^{-1}, $k_5 = 300$ sec^{-1}, and $K_q = 0.30$μM. *d*. $k_3 = 1.5 \times 10^9$ M^{-1} sec^{-1}, $k_5 = 300$ sec^{-1} and $K_q = 0.20$ μM. For other constants see Table I. (Ordinates as in Fig. 1.)

Tetani

As planned, repetitive stimulation produced prolonged and larger contractions.

When the interval between stimuli was 15 msec, the early part of the tension curve showed an inflection for each stimulus. At 10 msec intervals, the tension curve was smooth and rising faster. The tension rose still faster as the interval was shortened to 8, 5, and 2.5 msec. In fact, there would have been no limit to this, because a refractory period was not built into the model.

As soon as I tried tetani using the original excitation-contraction coupling assumption (equation 16), I realized that the simulation was inadequate, because the intramyofibrillar Ca did not reach a steady-state level during stimulation. Moreover, the area under the tension vs. time curve was directly proportional to the number of stimuli, a result contrary to experiment (16).

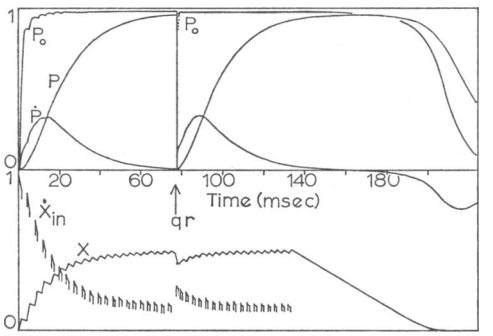


FIGURE 8 A simulated short tetanus, and a quick release (qr) with redevelopment of tension using the constants of Table I. Ordinates: P_o and P ($\times 1$); $\dot{P}$ ($\times$ 0.1 msec^{-1}), X ($\times$ 25μM), and $\dot{X}_{in}$ ($\times$ 3.3μM/msec). For clarity only the tops of the pulses of Ca flux are shown.

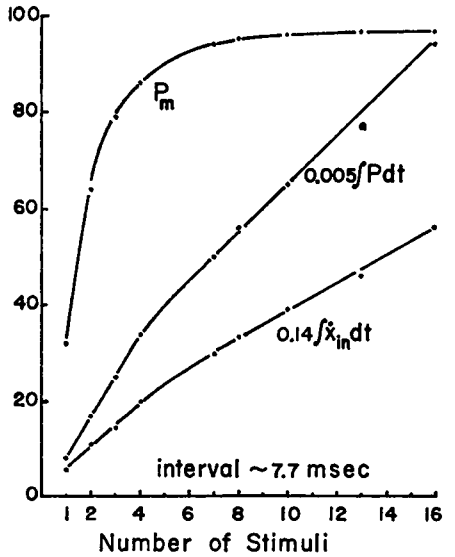


FIGURE 9 The dependence of the maximum achieved tension, the tension-time integral, and the total injected Ca upon the number of stimuli given at constant frequency (130 Hz).

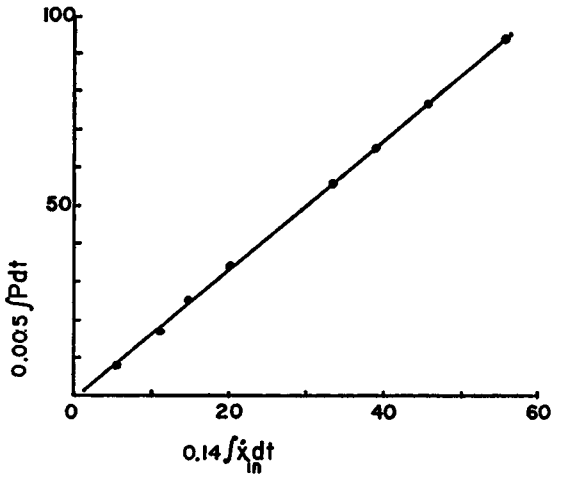


FIGURE 10 The direct proportionality of the time-tension integral to the total injected Ca.

Fig. 8 shows a tetanus (and a simulated quick-release experiment), using the extended assumption that the Ca influx was dependent on the level of Ca available to the muscle machinery. Fig. 9 shows the dependence of the maximum tension, the area under the tension curve, and the total injected Ca upon the number of stimuli applied. The curve of $\int_0^\infty P\,dt$ now has the bend seen in the reported experimental curves (15). The point for 13 stimuli is low probably because the average interval was greater than for the other contractions. The fact that the total injected Ca was also low suggested that $\int_0^\infty P\,dt$ was directly dependent upon the total injected Ca. Fig. 10 shows that this surmise was correct. The point for the 13 stimulus tetanus is now on the line.

It is significant that Jöbsis (16) gets a direct proportionality between the tension-time integral and the integral under the curve of fluorescence decrease attributable to mitochondrial NADH. Since this last is a measure of the energy flux through the system because of the contraction, it seems reasonable that it should depend upon, or be proportional to the work done by the pump, and hence upon how much Ca there was to be pumped away.

Summation

The summed effect of two stimuli, when the second occurs after relaxation has begun, results from the fact that the series elastic element is still stretched. Fig. 11 shows that in such a case, the second cycle of the active state is identical with the first. The reason is that by the time the tension is maximal, the active state has declined a long way (at room temperature at any rate, because the twitch maximum is only about one-third the tetanus maximum) and the available Ca is perhaps 5% of its maximum. When (as Fig. 11 shows) the second stimulus occurs at, or before, the tension maximum, the second cycle of the active state reaches a higher maximum.

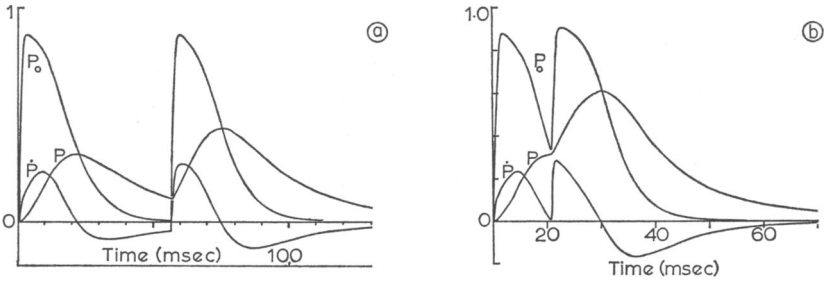


FIGURE 11 The summation of two stimuli: (*a*) second stimulus during relaxation of twitch, when cycle of P_o is practically complete; (*b*) second stimulus earlier, before first cycle of P_o is complete. Ordinates: P_o and P ($\times 1$), $\dot{P}$ ($\times$ 0.1 msec^{-1}).

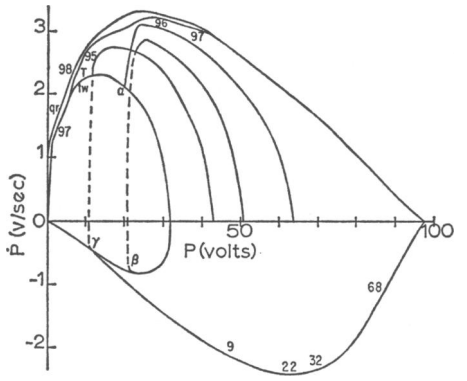


FIGURE 12 Phase-plane diagram showing a twitch (tw), three double stimulus experiments (tw + α, β, or γ), a tetanus (T), and the redevelopment of tension following a quick release (qr). The figures around the outer curves give the magnitude of the active state (in volts), throughout the contraction and relaxation phases of the tetanus and qr experiment.

However, even though the active state is usually the same in both cycles, its temporal relation to P and $\dot{P}$ is altered. This can usefully be shown by means of a phase-plane plot as in Fig. 12, where three double stimuli curves are compared with a normal twitch, a tetanus, and the tension developed after a quick release. The three curves lie between the twitch and the tetanus and, thus, in the double stimulus experiment the active state is never as great as in a tetanus. When compared on the basis of equal magnitudes of P, not t, the active state in the second cycle of the double stimulus experiment is larger, except for the first few milliseconds, than in the first cycle. This result agrees with the observations of Close (13). The fact that the active state cycles in summed twitches are not as large as in a tetanus or in the contraction developed after a quick release during tetanic stimulation (this gives the largest active state), has a bearing upon the interpretation of several methods for determining the time course of the active state (11, 12).

Redevelopment of Tension after a Quick Release

Jewell and Wilkie show a record of the redevelopment of tension after a quick release (4) beside the initial development of tension in the same muscle. The rede-

velopment occurs at a greater rate than in the original contraction. When they calculated the time-tension curve from separate determinations of the elastic properties and the force-velocity relation on the same muscle, they found that the calculated time-tension curve rose more rapidly still. It is clear from their calculated curve that $P(t = 0)$ is not zero, while for the experimental records it is. I estimate from them that $\dot{P}_m$ occurs at 25 msec in the original contraction, and at 10 msec in the redeveloped tension (2°C).

I first attempted to simulate this experiment by forcing P to be zero in the middle of a tetanus and then releasing it, all the while leaving all other variables unchanged. This was done by momentarily zeroing the integrator whose output is P, and then permitting it to resume integrating. Thus, the active state, P_o, was not changed in this "quick release." As could be predicted from equation 4, $\dot{P}$ underwent an instantaneous jump from zero to BFP_o/A when P was released from zero. Since this is contrary to experiment I made a further assumption.

For the second attempt I assumed that during a quick release the active state is also degraded to zero. Mathematically this ensures that when the redevelopment of tension begins $\dot{P}$ increases continuously, not discontinuously, from zero. It is also reasonable in terms both of the assumption that the concentration of Ca-M represents the active state, and of the sliding filament model of muscle. In a quick release all cross bridges would be broken and this surely would mean that every Ca-M complex is broken up.

The result of this assumption is shown in Fig. 8. The simulation is not quite as intended, in that the Ca concentration during the quick release decreases somewhat, whereas if anything it should increase because of the breaking up of Ca-M. This effect was an inadvertent result of the computer program designed to force P and P_o to zero and then release them. Nevertheless, the effect on $\dot{P}$ and P is as desired. (It was not worth repeating the simulation, because the false change in Ca concentration would not have had much effect on $\dot{P}$ and P.) The behavior of P_o is noteworthy. It rises up to its former value very rapidly indeed, exactly because the Ca level is high, some six times higher than the level in the first few milliseconds of the original contraction. Whereas it took 2.0 msec to reach the knee of $\dot{P}$ in the original contraction and 2.5 msec to the first maximum of P_o, in the redevelopment of tension it took about 0.4 msec to the knee and 0.5 msec for the active state to reach its former level. The difference between these figures probably accounts for the difference in the time of occurrence of the maximum in $\dot{P}$, viz 12.2 and 10.5 msec.

While the simulation of a quick release experiment has made it clear that one need not suppose the active state to be unchanged by a quick release, it has not thrown any light on the discrepancy reported by Jewell and Wilkie (4) between the calculated and measured myograms. The tension did not rise perceptibly faster in the redevelopment when the active state was supposed unchanged. Perhaps a difference between the two simulations could be seen if the computation was carried out with constants appropriate to muscle at 2°C. This has not been tried.

The P and $\dot{P}$ curves of Fig. 8 are replotted against each other in Fig. 12. From such an experimentally obtainable plot one can infer that the active state is still rising to its "plateau" during the first few shocks of a tetanic stimulation. For this model, Fig. 8 shows that this is the case. Fig. 1 of Close (13) yields similar information about a twitch at 0°C.

DISCUSSION

A. The key assumption of this model, which permits a theory of excitation-contraction coupling to be linked with an analysis of muscle contraction, is that the magnitude of the active state is proportional to the concentration of activator-muscle complex, and as a consequence, its time course is the kinetic course of this complex. Relaxation is made possible by including a simple carrier pump to remove the activator. Through study of the model, I was forced to make a small extension to Sandow's theory of excitation-contraction coupling, so that the inflow of activator depends upon a membrane permeability controlled by the membrane potential as well as upon the myofibrillar concentration of activator.

B. The model is pleasingly successful in reproducing (on the correct time scale and in largely correct proportions) the isometric tension curve, P, and its time derivative, $\dot{P}$, including the knee (or hump (7)) of single twitches, and in addition the following well known phenomena: potentiation (A and B type), tetanus, redevelopment of tension after a quick release, and the summation of single twitches. An examination of the representation of this last effect, leads to the same conclusion regarding the active state as Close reached from an experimental study (13); namely, that the active state does not reach its absolute maximum near the beginning of a twitch or a tetanus. The explanation of this in the model differs from Close's and will be discussed in connection with the latent period.

C. The nearest experimental equivalent of this model is a single fibre preparation where isometric studies are referred to a fixed sarcomere length, (for example, Edman's (12)) and where stimulation is massive (15) and supermaximal. This point should be kept in mind in assessing the model and using it for predictions.

D. Models are never complete. In this model I have ignored the existence of the latent period and the involvement of adenosine triphosphate (ATP). How serious are these omissions? For ATP it is enough to suppose that it is present in sufficient excess, so that any variations in its concentration because of activity does not affect any of the rates in the model. This appears a not unreasonable assumption for twitches and short tetani. It appears that one can probably account for the latent period of 2 msec at room temperature, (17, 18) in terms of the inward spread of depolarization along the T-tubules and the diffusion of the calcium to its site of action. For the model this means that the part of the latent period that is pure time delay can be ignored without loss, while there must be a temporal spread in the instant of activation of about a millisecond. The effect of such a temporal spread

has been worked out by Close (13) and used to explain why the active state of a muscle takes some time to reach its tetanus maximum, while the maximal velocity of shortening is achieved much sooner. A temporal spread in activation of 1 msec would lead to a slight smearing out of the curves presented here, but would not seriously affect the conclusions drawn.

Close's result and his explanation (13) are worth further discussion. As mentioned before, his phase-plane plot leads to the same conclusion as the comparison in Fig. 12 of a tetanus with the redeveloped tension after a quick release: that it takes some time for the active state to reach its absolute maximum. He poses three alternative explanations: (*a*) "Synchronous activation of all contractile units," with purely temporal delay in the rise of the active state. (*b*) "Longitudinal gradation of activity"—as the activation wave passes lengthwise along the fiber each unit comes instantaneously to full activity. (*c*) "Transverse gradation of activity"—activation sweeps inward from fiber surface bringing units instantaneously to full activity as it passes. The third explanation is very successful in accounting for his results. The present model gives a similar observable result based on explanation (*a*). Close dismissed (*a*) because of the early achievement of maximal velocity of shortening. His argument is correct, but is not decisive because his tacit assumption—that the active state would not reach 0.90 of its maximum in a tetanus very rapidly indeed and then would take much longer to achieve 0.98—is not necessarily true. Clearly, the active state of the model will give a maximal shortening velocity of $0.88v_{max}$ about 1 msec after shortening begins, but the active state will not reach 0.98 until 25 msec have elapsed—the exact time depends on the stimulation rate. Because experimental records are the sum of many units, explanations (*b*) and (*c*) are valid and are currently employed to explain the latent period (17, 18). The model, however, demonstrates that (*a*) adequately covers observed phenemena, apart from the latent period.

E. What is the effect of simplifying assumptions used in adapting the model to the computer? (1) The use of equation 2 for values of P above $0.5P_o^{max}$: for normal and potentiated twitches and for the double stimulus experiment there will be no effect because P does not rise above $0.5P_o^{max}$. Use of this relation will definitely increase the rate of rise of tension in tetani in the latter part of the contraction phase, and affect the right hand portion of phase-plane plots accordingly. I do not believe this simplification undermines any of the conclusions drawn in this study.

(2) If one follows the electrotonic theory of the spread of membrane depolarization inward along the T-tubules (18), it seems unlikely that the time course of the Ca flux is given by the action potential itself, as assumed here. However, because this event is over before the active state is very large, the significant events depend less upon the profile, and more upon the total amount and upon the kinetics of binding and pumping after that.

(3) The pump kinetics are the simplest possible. Nothing is gained by elaborating the carrier type mechanism, as the basic steady-state rate law would be unchanged. Alterations in the rate law of the pump, such as would arise from a different mecha-

nism, are well worth considering and merit a separate study. By introducing a degree of inhibition at higher Ca concentrations, analogous to substrate inhibition, the relaxation phase might agree better with reality. By considering the pump rate to be influenced by how much Ca is stored by it in a temporary location (as in the longitudinal tubules (19)), such phenomena as the staircase effect and post-tetanic potentiation could be looked for in the model, and perhaps, by working backwards, inferences drawn about the rates of Ca transport between the various compartments.

F. A good model can explain more than it was designed to explain. Here are examples where the present model predicts or explains an observed result: (*a*) Since the knee in the $\dot{P}$ trace reflects the time of occurrence of the first local maximum in the active state, and this depends upon the kinetics of Ca binding by the muscle machinery and not upon the Ca influx as long as this is rapid enough, we expect the time of occurrence of the knee to be unaffected by potentiation. This is what Sandow and Brust (7) report for caffeine potentiation. (*b*) In the model the higher the rate of stimulation the less the amount of Ca injected per twitch. If ATP breakdown is, *inter alia*, stoichiometrically associated with pumped Ca, then Davies' results (20) are to be expected, that in a series of twitches and short tetani less ATP is split per pulse at higher stimulation frequencies. (*c*) By the time P_m is reached in a twitch only about 5% of the free Ca remains. This must be so because at this moment the active state is about half-maximal and, thus, the ligand must be at much less than a tenth its concentration at the maximum of the active state. This explains the otherwise puzzling observation of Jöbsis and O'Connor (21) on murexide treated toad muscle that virtually all the Ca released on excitation had disappeared by the time of the twitch maximum. In addition, their observations with four stimuli agree with point (*b*) above. The elegant experiments of Ashley and Ridgway (22) on single muscle fibres from *Balanus nubilus* also agree with the expectations of the model. (*d*) Close's relation (23), that the product of the contraction time of a twitch and the maximal speed of shortening of an average sarcomere is a constant, can be derived from the kinetics of the model as an approximation. The constancy of the product means that the product of total injected Ca per stimulus and total number of binding sites, per sarcomere, and maximum pump speed remains constant. The magnitude of the constant should therefore increase with potentiation.

G. Because of the model's success in fitting or explaining a wide range of phenomena associated with isometric contraction, I conclude that it can be used as a reliable guide in restructuring our notions of the active state, as a basis for criticizing some methods of determining the time course of the active state, and as a source of hypotheses for further experimental test.

The model contributes little concerning the decline of activity since it takes over the general kinetics that have been reported (8, 11), except in showing that the pattern of decline can arise from a very simple underlying chemical mechanism. The

interest centers on the early stages of activity, because here experiments do not give unequivocal answers. The model makes it clear that isometric contraction phenomena do not require that the active state reach the same maximum in each kind of experiment. Thus, in twitches the maximum is about 0.88 of the absolute maximum. If a second stimulus is given when the twitch tension has begun to decline, the second maximum in the active state is scarcely larger. When, however, the second stimulus comes at or before the twitch maximum, that is, before the active state from the first stimulus has fallen to half its maximum (see Fig. 11), the next maximum is perceptibly higher. This is because the summation of active states occurs *via* the myofibrillar Ca concentration. The same pattern is followed in a tetanus (Fig. 8). After each stimulus the active state rises to a new maximum, starting with 0.88 (the actual value depends on the parameters of the model, Table I) and rising in an unfused fashion toward 1.0. The time course may be described as biphasic: a rapid rise to the first maximum, requiring around 1 msec at room temperature, marked by the knee or hump in $\dot{P}$ (7), followed by an oscillatory phase, in which a rising series of maxima approach the limit 1.0. The time required for successive maxima to reach this limit will depend upon the stimulus frequency. This oscillatory behavior will occur even though the tension curve is fused. It should, however, be experimentally observable in the $\dot{P}$ trace. In sum, in a twitch the active state does not show a "plateau" or reach the same maximum as in a tetanus. It is still appropriate to term the broad maximum reached in a tetanus, a "plateau" even though it is not perfectly flat.

From what has been said about the dependence of the active state kinetics on the pattern of stimulation, it should be clear that Ritchie's double stimulus method (11) and Edman's multiple stimulus method (12) will both over-estimate the magnitude of the active state for values much above 0.5.[3] This is very apparent when I apply Edman's method to data from the model. Servo-controlled quick stretches in a preparation such as Edman's, where the sarcomere spacing can be monitored, might be the most accurate way of measuring the time course of the active state after a single shock. Ritchie's own data bear out my contention (11).

Wilander's (24) observations on rat gastrocnemius muscle are quite compatible with this model. It is, therefore, not necessary to postulate an "active state for shortening" and an "active state for tension maintenance." He finds the fusion frequency for isotonic shortening to be about twice that for isometric contraction. The model shows analogous behavior. Small changes in the active state, P_o, appear as proportional changes in both velocity and $\dot{P}$, but P can show only their integrated effect. In the model the fusion frequency for tension is between 75 and 100/sec, while at 191/sec $\dot{P}$ is not fused until the fourth stimulus. Even at 385/sec $\dot{P}$ is unfused for the first three shocks.

[3] Nevertheless, I am pleased to note, as Edman has pointed out privately, that the results of his experiments (12) seem to agree very well with the calculations I have presented.

Since it follows very simply from equation 4 that

$$\int_0^\infty P\,dt = \int_0^\infty P_o\,dt \qquad (22)$$

we can understand that the time-tension integral, whose usefulness has been shown by Jöbsis (16), is a direct measure of the corresponding area under the curve of bound Ca vs. time, which area can be expected to relate rather directly to the area under the curves of other chemical intermediates.

A good model should suggest further experiments as well as refine our perception and our interpretation of existing experiments.

The model makes it possible to work out what results could be observed from specific interference, either with excitation-contraction coupling or with the pump in vivo. Or by working backwards from the staircase effect one should be able to state the nature and time course of changes in the pump.

A specific prediction is that the fluctuations in $\dot{P}$ will disappear after fewer stimuli the higher the rate of stimulation. Coupled with Jöbsis' murexide method of demonstrating the concentration changes in free Ca the diminution in the fluctuations could be used to follow the titration of the muscle sites that bind Ca.

The following experiment should provide a check on the underlying postulate of this model. Measure the injected Ca and the tension in clamped depolarizations.

Finally, a small but possibly useful prediction. Although potentiation is not demonstrable in a tetanus, or at low temperatures where the twitch/tetanus ratio approaches 1, the effect will still be demonstrable in the early phase of the $\dot{P}$ trace.

I am most grateful to Dr. A. Sandow for his infectious enthusiasm and stimulating criticism. He may not know how much the completion of this study owes to his interest.

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REFERENCES

1. SANDOW, A., S. R. TAYLOR, and H. PREISER. 1965. *Fed. Proc.* **24**:1116.
2. HILL, A. V. 1953. *Proc. Roy. Soc. Ser. B. Biol. Sci.* **141**:498.
3. SANDOW, A. 1961. Biophysics of Physiological and Pharmacological Actions. A. M. Shanes, editor. AAAS, Washington, D. C. 413.
4. JEWELL, B. R., and D. R. WILKIE. 1958. *J. Physiol.* **143**:515.
5. SANDOW, A. 1958. *Science.* **127**:760.
6. WEBER, A., R. HERZ, and I. REISS. 1964. *Fed. Proc.* **23**:896.
7. SANDOW, A., and M. BRUST. 1966. *Biochem. Z.* **345**:232.
8. JEWELL, B. R., and D. R. WILKIE. 1960. *J. Physiol.* **152**:30.
9. PODOLSKY, R. J., and L. L. COSTANTIN. 1964. *Fed. Proc.* **23**:933.
10. CHANCE, B. 1943. *J. Biol. Chem.* **151**:553.
11. RITCHIE, J. M., and D. R. WILKIE. 1955. *J. Physiol.* **130**:488.
12. EDMAN, K. A. P., D. W. GRIEVE, and E. NILSSON. 1966. *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **290**:320.
13. CLOSE, R. 1962. *J. Gen. Physiol.* **46**:1.
14. MACPHERSON, L., and D. R. WILKIE. 1954. *J. Physiol.* **124**:292.

15. SANDOW, A., and H. PREISER. 1964. *Science*. **146**:1470.
16. JÖBSIS, F. F., and J. C. DUFFIELD. 1967. *Science*. **156**:1388.
17. SANDOW, A. 1965. *Pharmacol. Rev.* **17**:265.
18. FALK, G. 1968. *Biophys. J.* **8**:608.
19. WINEGRAD, S. 1968. *J. Gen. Physiol.* **51**:65.
20. DAVIES, R. E., M. J. KUSHMERICK, and R. E. LARSON. 1967. *Nature*. **214**:148.
21. JÖBSIS, F. F., and M. T. O'CONNOR. 1966. *Biochem. Biophys. Res. Commun.* **25**:246.
22. ASHLEY, C. C., and E. B. RIDGWAY. 1968. *Nature*. **219**:1168.
23. CLOSE, R. 1965. *J. Physiol.* **180**:542.
24. WILANDER, B. 1966. *Acta Physiol. Scand.* **68**:1.