ACTIVE STATE OF MUSCLE IN IODOACETATE RIGOR*

By GEORGE E. MAURIELLO‡ AND ALEXANDER SANDOW

(From the Department of Biology, Washington Square College of
Arts and Science, New York University, New York)

ABSTRACT

Frog sartorius muscles, equilibrated to 2×10^{-4} M iodoacetic acid–Ringer's solution and activated by a series of twitches or a long tetanus, perform a rigor response consisting in general of a contractile change which plateaus and is then automatically reversed. Isotonic rigor shortening obeys a force-velocity relation which, with certain differences in value of the constants, accords with Hill's equation for this relation. Changes in rigidity during either isotonic or isometric rigor response show that the capacity of the rigor muscle to bear a load increases more abruptly than the corresponding onset of the ordinarily recorded response, briefly plateaus, and then decays. A quick release of about 1 mm. applied at any instant of isometric rigor output causes the tension to drop instantaneously to zero and then redevelop, the rate of redevelopment varying as does the intensity of the load-bearing capacity. These results demonstrate that rigor mechanical responses result from interaction of a passive, undamped series elastic component, and a contractile component with active state properties like those of normal contraction. Adenosinetriphosphate is known to break down in association with development of the rigor active state. This is discussed in relation to the apparent absence of ATP splitting in normal activation of the contractile component.

INTRODUCTION

A muscle poisoned with iodoacetic acid (IAA) suffers a series of well known metabolic disturbances which cause it to go into rigor (1–3) and thus shorten and develop tension. The rigor effects develop most strikingly when the IAA-treated muscle is first subjected to a burst of normal activity, such as a long tetanus or a series of twitches. But, as shown previously, especially in a paper from this laboratory (4), the essential mechanical features of rigor appear, though less intensely and on a slower time scale, in a poisoned muscle that has not been excited to perform contractions at all. The general study of these results led to the inference (4) that the mechanical effects of rigor develop in

consequence of active state processes qualitatively like those of normal muscular contraction. The present work, previously reported in preliminary form (5), tests this inference by studying the mechanical responses of rigor muscle in certain special experimental procedures known to reveal the basic active state mechanisms of ordinarily contracting muscle.

Mechanical activity of a normal muscle results from the interaction of its contractile component, in which excitation causes generation of the active state, and an elastic component in series with the contractile, which is undamped and non-linear, and is not transformed by stimulation but acts passively as would any equivalent spring (6–9). The active state is defined by two fundamental properties of the contractile component: (a) the capacity to shorten in accordance with a hyperbolic force-velocity relation, this being shown directly by the dependence of speed of isotonic shortening of whole muscle on the load it must lift; and (b) the ability to bear a load; i.e., a rigidity, which at greatest is given by the maximal tetanus tension (P_0) developable by the muscle. In general, the intensities of these two properties, and thus the active state intensity, vary together as a function of time following the initiation of activation by stimulation. Detailed tests prove (10) that the active state develops to full intensity very abruptly, and, after maintenance at maximum for a short period, it then gradually decays to zero. The mechanism of interaction of this active state response with the compliance of the series elastic component need not be detailed here. But the presence and certain properties of the elastic material are most clearly indicated by the very sudden disappearance of tension of a fully activated muscle subjected to a small quick release (11, 12).

Our work demonstrates that the mechanical behavior of muscle in IAA rigor is attributable to active state mechanisms qualitatively like those of normal muscle. The finding that the contractile component of the muscle in rigor has characteristic active state properties suggests that the mechanochemistry of rigor development is similar to that of normal contraction. Thus, by taking into account known chemical changes of rigor, our results indicate certain mechanochemical features of this kind of muscular activity that may be of value in determining the nature of contraction in general.

General Methods

Sartorii of the frog (*Rana pipiens*) were excised, soaked for 1 hour in oxygenated, phosphate-buffered (pH 7.2) Ringer's solution containing 2×10^{-4} M IAA, and then mounted in a chamber and connected by a fine metal chain to either an isotonic or isometric lever as described below. The initial tension was always adjusted (generally at 0.75 gm.) to set each muscle at its standard rest length so as to avoid complications by parallel elastic elements in the production and interpretation of the results. The muscles were then subjected to a burst of electrically activated contraction consisting of a series of twitches or a long tetanus and tested for some particular active state property during the ensuing rigor response.

In experiments involving measurement of shortening or extension, the muscle was mounted in a moist chamber, and length changes were recorded kymographically by an inertialess isotonic lever. Stimulation was effected by maximal shocks of 0.2 msec. time constant from a conventional thyratron-controlled stimulator, and they were applied by wire electrodes placed near the ends of the muscle.

In the isometric tests tension changes were recorded by means of the RCA Type 5734 mechanoelectronic transducer tube and D. C. cathode ray oscillography. The relevant tension alterations ran their course over a time interval of several minutes. Correspondingly long oscillographic sweeps were provided by a phantastron sweep-generator like that described by Dickinson (13). This device was made to produce sweeps of up to 5 minutes' duration, and excellent linearity, by suitably increasing (up to 40 sec.) the time constant of its cathode-follower-controlled R-C timing circuit. The isometric muscles were mounted totally immersed in Ringer's solution and massively stimulated by maximal 0.2 msec. square-wave shocks generated by a special high current stimulator (14). The difference in technique used for stimulation of these and the isotonic muscles is of no essential importance; it was due merely to differences in availability of apparatus at the time of performance of the particular types of test.

All experiments were done at or near 25°C. Special procedural details will be presented later in relevance to the particular kinds of experiments performed in this work.

<div align="center">RESULTS</div>

Force-Velocity Relation.—In experiments of this type on normally contracting muscle, all the required data for determination of a force-velocity curve, and, from this the relevant fundamental dynamical constants, are obtained by means of a set of tests done on a single muscle (6). Such procedure was impossible in our work since any muscle could be put through a rigor response only once. It was therefore necessary to use pooled results from many muscles and then subject them to statistical analysis. In one series (isotonic) the rigor shortening speed of each muscle was obtained at some particular load; in another series (isometric), each muscle was tested for its maximal rigor tension, P_{OR} (the equivalent of the normal P_0). Furthermore, account had to be taken of the fact that P_{OR} varies with the kind of conditioning activity that provokes generation of rigor; *e.g.*, a prolonged tetanus causes a greater maximal rigor tension than does a twitch series (4). But tetanically engendered rigor could not be used because, in isotonic experiments (unless the load is relatively large), the earliest phase of rigor shortening is masked by the terminal fatiguing part of the tetanus. This masking effect was avoided by using as conditioning activity a 1/sec. series of maximal twitches and terminating this as rigor shortening just became evident (or was about to begin as judged by reduction to zero of the lever overshoot (see Fig. 1 *a*)). A consistent value of P_{OR} had then to be determined (isometrically) by a similar activation procedure. Earlier work (4) had shown that following a twitch series $P_{OR} = 12$ to 14 gm. In
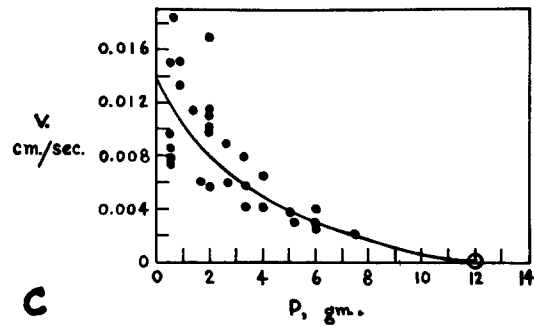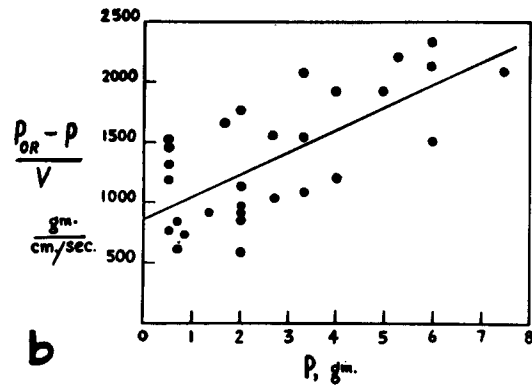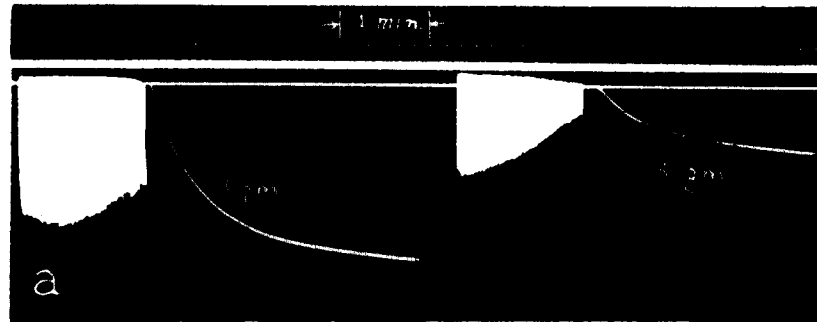
FIG. 1. Force-velocity relation of muscles shortening in IAA rigor. (a) Two typical records at indicated loads of 1 and 3 gm.; in each, the smooth curve represents the rigor shortening as a function of time and the preceding fused traces indicate the 1/sec. series of conditioning maximal twitches causing the muscle to develop rigor. (b) Linear plot of load-velocity data obtained from twenty-nine different muscles. See text for details. (c) Usual hyperbolic plot of the same data.

the present work it has been found that $P_{OR} = 12 \pm 1.2$ gm., and this is the value we use in our analysis of the force-velocity data.[1]

Representative records of these experiments, shown in Fig. 1 a, demonstrate that, as in normal contraction (6, 15), rigor shortening occurs at a lower speed under a greater load, and that the speed is maximal at onset of each shortening and remains quite constant for a certain early portion of the response. This period of linear shortening under each load provides us with the velocity data needed for this work. A complete compilation and analysis of the data obtained from twenty-nine muscles shortening under eleven different loads are presented in Fig. 1 b and 1 c. For analysis we have used the method of Shapiro quoted in the work of Katz (16). Thus, Hill's equation (6) is transformed (and symbols for all constants rewritten to correspond to the state of rigor) to read: $(P_{OR} - P)/v = (1/b_R)P + a_R/b_R$, which is the equation of a straight line, when $(P_{OR} - P)/v$ as ordinate is plotted against P as abscissa, with ordinate intercept $= a_R/b_R$ and slope $= 1/b_R$. In these relations: P(gm.) is the load and v(cm./sec.), the corresponding speed of shortening; P_{OR}(gm.), the relevant maximal rigor tension $= 12$ gm.; and a_R(gm.) and b_R(cm./sec.) are constants to be determined from the values of the mentioned intercept and slope. Fig. 1 b gives all our data plotted in this form, with the straight line, obtained by the method of least squares, as the best fit to the rather widely scattered points. The intercept and slope of this line are, respectively, $a_R/b_R = 880$ and $1/b_R = 180$. Thus $b_R = 5.4 \times 10^{-3}$ cm./sec., and $a_R = 4.8$ gm., or since $P_{OR} = 12$ gm., $a_R/P_{OR} = 0.40$. Fig. 1 c presents our results in the more usual form (6), the hyperbolic plot of the force-velocity relation: $(P + a_R)(v + b_R) = b_R(P_{OR} + a_R)$; and it is evident that the smooth line calculated by use of the determined values of the constants represents fairly well the average positions of the experimental points.

In view of the large scatter of the data of Figs. 1 b and 1 c, we recognize that our results have validity only within a certain statistical framework,[2] and

---

[1] Our procedure omits normalization of the various rigor outputs in respect to length, mass, or other parameters of the different muscles. We considered it unprofitable to attempt such normalization in general since any resultant reduction in scatter of results would certainly have been lost in the very much larger scatter that we know to be characteristic of the values of any parameter of IAA rigor behavior. In any case, technical difficulties prevented relating any pair of P-v data to the P_{OR} output of a given muscle since that muscle could be used only once, and thus only for either of these kinds of results. Precautions were taken, however, to reduce variability among the muscles by choosing them to be of fairly uniform length and mass. Furthermore, in order to ensure validity of the statistical analysis of the results (see later), muscles tested in particular P-v determinations were chosen at random with respect to their individual lengths and weights.

[2] The statistical justification for our conclusion that shortening in rigor is like that

that our calculated values of a_R and b_R are quite rough estimates of these parameters for the rigor muscle. However, it is noteworthy that our value of $a_R/P_{OR} = 0.40$ is not too different from the corresponding normal value, $a/P_O = 0.25$; and as for b_R being so small in relation to the normal $b = 1$ cm./sec., this is consistent with the fact that shortening speed in rigor is much less than that of normal contraction.

However, the main point of this analysis is that it strongly indicates that the shortening of the contractile component of a muscle in IAA rigor occurs in accordance with a hyperbolic relation between force and speed, and thus this component is in an active state like that of normally activated contractile elements. Furthermore, by taking into account the variation in speed of shortening in each rigor response (see Fig. 1 a), and recalling that in normal contraction shortening rate is a measure of active state intensity, our results indicate that the rigor active state, like the normal one, develops abruptly to maximal strength, remains there for a brief period, and then decays.

Elasticity Changes.—In these experiments muscles were activated to develop rigor isotonically by a procedure like that in the force-velocity work, except that (apart from necessary changes noted later) the afterload was always the same as the initial load (0.75 gm.). Muscles so treated perform a rigor sequence consisting in order of the following phases: (a) fast shortening (several minutes), (b) progressively slower shortening (about 45 minutes), (c) plateau (about 15 minutes), and (d) relaxation (1 to many hours). As each muscle progressed through such an output, it was tested for extensibility at a more or less arbitrarily chosen point of each of these phases of rigor. In addition, such tests were made on the poisoned muscles at rest just before they were

---

in ordinary contraction is based on showing that the relation between P and v in rigor is hyperbolic. This depends on the validity of our least squares determination of the straight line of Fig. 1 b. In particular, it may be asked whether there is a reasonable chance that such a straight line has a zero slope; for, if this were so, we could have $(P_{OR} - P)/v =$ constant, and thus the corresponding relation between P and v would be linear, and not hyperbolic as in Fig. 1 c. Using our data of Fig. 1 b and the "t-test" method given by Dixon and Massey (17, p. 160), we determine the probability that the questioned line would have zero slope is less than 0.005. This is so small that we reject the assumption of zero slope. Hence we infer that the least squares straight line of Fig. 1 b is a reasonably correct estimate of the regression of the points for $(P_{OR} - P)/v$ on P, and that, therefore, the P-v relation for rigor is hyperbolic. Furthermore, using another test described by Dixon and Massey (p. 158), we find that at the 90 per cent confidence limits the slope of our linear regression line will fall in a range from 242 to 126. Since b_R equals the reciprocal of this slope, we would expect that at the same confidence limits b_R would vary between 0.0041 and 0.0079 cm./sec. No similar test for a_R has been made. But, in any case, we consider that our determined value for this constant, as for that of b_R, can only be a rough estimate of what is true for any particular case of shortening in IAA rigor.

subjected to the twitch activity series. Each extensibility test was made by
subjecting the muscle for a period of about 15 sec. to an extra load of 1 gm.
(in a few tests, of 2 gm.) and recording the corresponding extension on the
kymograph. The extra load was then removed and the muscle proceeded in its
rigor development under the standard afterload until the next extensibility
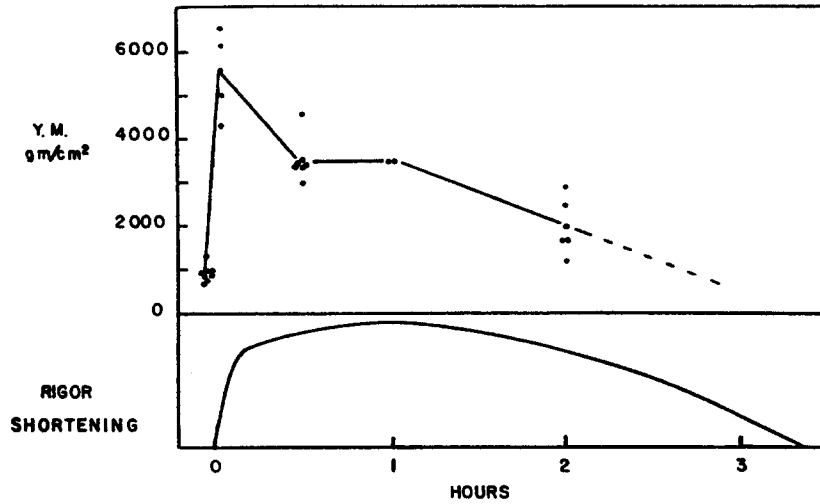test was made by again momentarily subjecting the muscle to the extra load.

FIG. 2. Variations in Young's modulus of muscles in relation to the phases of an
isotonic rigor response. The lower smooth curve is a generalized trace corresponding
to the average behavior of muscles in rigor responses under an initial and afterload of
0.75 gm. Note sequence of phases: rapid and then slow shortening, short plateau, and
final, prolonged relaxation. The dots on the graph give values of the modulus of elas-
ticity for states of the muscle as follows: the first set (extreme left) for the IAA-treated
muscle at rest (before the conditioning twitch series, which is not shown) and the
successively following four sets obtained during the respective phases of fast shorten-
ing, plateau, and relaxation.

The paired load-extension data so obtained were used to calculate Young's
modulus (E) by means of the usual formula, $E = (\Delta F/A)/(\Delta L/L)$, in which
ΔF(gm.) = the impressed increment of load, ΔL(cm.) = the corresponding
extension, L(cm.) = the muscle length at the time of the particular test, and
A(cm.^{2}) = the corresponding cross-sectional area. This area was calculated by
the formula, $A = M/dL$, (M = weight of muscle determined at the end of
an experiment, and d = its density which was taken as 1.05), under the as-
sumption that the muscle is a uniform cylinder defined by the values of L
and A.

Fig. 2 presents the results of these studies made on seven different muscles,

and they demonstrate that the rapid transition from rest to the fast phase of rigor involves a seven-time increase in the elasticity coefficient. Thereafter, as the more sluggish rigor phases run their course, the value of E falls in a more or less progressive fashion. Thus, in a rigor sequence, as in the normally activated contractile cycle, the rigidity of the muscle, *i.e.* its load-bearing capacity, greatly and abruptly increases at first and then more slowly decreases. This is further evidence indicating presence of an active state of the contractile component in a rigor response, whose intensity, moreover, has a characteristic temporal variation.

Effects of Quick Release.—In these experiments the muscle was isometrically connected to the transducer tube myograph and its chamber was clamped on a rack and pinion that was operated by hand to effect quick releases of the muscle. Hand operation was rapid enough since the rigor changes were relatively very slow. There was no automatic provision for stopping a release at a predetermined amount, nor was this desired since in view of the variability of the rigor output there was no way to know beforehand how big any release should be to achieve the desired immediate effect; *i.e.*, a drop of the existing rigor tension to zero. Furthermore, in the experiments involving a series of releases applied at successive moments of a rigor response, automatic stopping would have introduced difficulties due to the need for resetting the stop in preparation for each new release of the series. Thus, what was done was to watch the movement of the recording spot on the cathode ray screen and to terminate the releasing operation when the tension was judged to have dropped to zero. The release effects in Fig. 3 show that this procedure was fairly successful, but, in any case, of sufficient precision for our present needs.

Fig. 3 *a* illustrates some typical results. The IAA muscle was first maximally tetanized until it was fully fatigued. No part of the tetanus contraction period is evident since the associated very rapid movement of the cathode ray trace resulted in complete underexposure of this part of the film. Nor is there any indication of the tetanus plateau since this was off screen. Thus the recorded tension changes begin with the slowly diminishing output of the fatiguing muscle represented by the downward coursing trace at the extreme left. When the tetanus tension had decreased to about 3 gm., onset of rigor occurred and the tension then developed along a sigmoid curve which is typical of this part of the response, reaching a plateau in about 15 sec. Left to itself, such a muscle maintains the plateau for at most a minute (present controls give an average of about 15 sec.) and then spontaneously relaxes during the ensuing 2 hours or so (4). But during the plateau of this experiment a quick release was applied which was of sufficient amount to cause a drop of tension to the zero rigor level. Tension then redeveloped at a rate which was highest at the start (*i.e.*, without the slowly rising foot of the original rigor development), and which progressively diminished as tension increased to a new but lower plateau. There
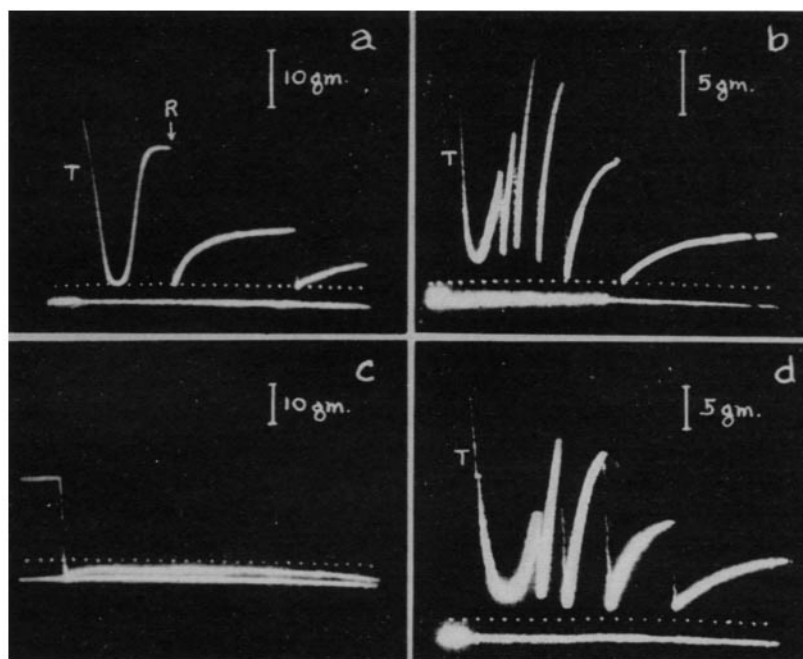
FIG. 3. Effects of quick release on tension of muscles at various phases of isometric rigor. Each record gives the behavior of a separate muscle. In the first record, R indicates the application of a 1 mm. release and the resulting very rapid and great fall in tension; in the other records similar tension changes were produced by similar applications of a release at the indicated moments of a response. The record of c was obtained from a muscle that was permitted to go through an undisturbed rigor response for 5 minutes, at which time it had lost 40 per cent of its plateau tension, and was then released; note the very slow and small redevelopment of tension. The dots give time in 5 sec. intervals, and the lowermost steady line of each record represents the initial tension (0.75 gm.) of each muscle. T indicates the terminal part of fatigue of the conditioning tetanus. Maximal tetanus output (P_O) of each muscle before IAA treatment, and corresponding maximal rigor output (P_{OR}) when this was permitted to be reached: (a) $P_O = 55$ gm., $P_{OR} = 27$ gm.; (b) $P_O = 50$ gm.; (c) $P_O = 65$ gm. $P_{OR} = 36$ gm. (recorded, but not shown); (d) $P_O = 54$ gm. For further details, see text.

then followed a second quick release with effects like those of the first, except that the redevelopment of tension occurred at a generally slower rate and reached a still lower plateau. In general, these results and those of Fig. 3 b, 3 c, and 3 d are like those of Aubert (18, 19). However, he used a uniform release of about 2.2 mm. in all his experiments which involved muscle rest lengths varying from 29 to 32 mm., and it is not clear whether this release was just

enough to cause a drop of tension to zero. Furthermore, his experiments involving redevelopment of tension following release are rather different from ours for he studied this process in muscles under forcible elongation to a greater length than they had before release.

Our results are of interest in two respects: (a) the immediate drop in tension at release; and (b) the subsequent rate of rise of tension. In analogy with the explanation of comparable changes in normal contraction (12, 20), the first of these effects is attributed to loss of tension in an undamped passive series elastic element. In a number of special experiments like that of Fig. 3 a, we have determined the release just sufficient to cause the tension at plateau of rigor to drop to zero: on the average this was 1 mm.; i.e., 3 per cent of the standard rest length (33 to 34 mm.) of the muscles, and thus quite as in normal muscle (12).[3] Our results are not detailed enough to tell whether the series elasticity is non-linear, but there is no reason to believe that in this respect rigor muscle differs from the normal.

The variations in rate of redevelopment of tension following release are of interest since, as in the comparably treated normal muscle (12, 21), this rate at any moment may be taken as a measure of the concurrent intensity of the rigor active state. We limit ourselves to considerations of this rate during the foot of the original rise of rigor and at onset of its redevelopment following each quick release, this rate being indicated by the corresponding slopes of the various tension-time curves given in the records of Fig. 3. Taking into account all the relevant recordings included in Fig. 3, we obtain a general picture as follows: the rigor active state intensity rises relatively abruptly for the short time of the foot, it plateaus at maximal value for about 15 to 20 sec., and then very gradually decays. This analysis is not completely unambiguous since the various quick release effects of each of the tests of Fig. 3 b were obtained on a muscle that was becoming shorter and shorter in consequence of the accumulated releases of the series; i.e., the rates of tension development we are comparing were obtained at different lengths of the muscle. However, essentially the same general result regarding variation in rigor active state intensity is obtained from other experiments such as those of Fig. 3 a, 3 c, and 3 d, in which the length changes due to release were not as extensive as those used in obtaining the results of Fig. 3 b. We therefore feel that the general outline of rigor active state kinetics given above is essentially correct. And thus these results again demonstrate that the active state intensity varies in a rigor re-

---

[3] This result must be qualified since the muscles had developed an average maximal rigor tension of about 57 per cent of that produced in maximal tetanus. Therefore, the stretch of the series elastic element in rigor would be less than that in tetanus. But, if the non-linearity of the stress-strain curve of the elastic element is unchanged by rigor, the mentioned difference in tension would involve a much smaller relative length difference, and thus the essential significance of the above discussion would still hold.

sponse as it does in a normal twitch or a short tetanus, though, of course, on a generally much slower time scale.

Effects of Quick Stretch.—These studies involved general procedures like those for quick release, but now the racking was limited by stops so that a controlled 2 mm. stretch (and release therefrom) could be impressed on the muscle. It was of interest to determine first whether stretches applied very early in a rigor response caused tension increases like those shown by Hill (10) to develop when a muscle is stretched very early in a twitch. For such tests it was inappropriate to condition the muscle for rigor production by a tetanus extended for such a long time (as in the tests of Fig. 3) that rigor begins before tetanus output terminates. Experiments were therefore done with a 4 sec. conditioning tetanus, after which the IAA muscle completely relaxes and remains so for about 1 minute (as if in a "latent period" of the oncoming rigor response), and then begins the overt mechanical changes of rigor. Such a rigor, furthermore, develops at a generally slower pace and this also facilitates use of the procedure of interest to us.

A typical experiment of this type is presented in Fig. 4 *a* and 4 *b*. Fig. 4 *a* demonstrates the effect on the IAA-treated but still resting muscle of a series of 2 mm. stretches and releases. Each stretch yields a tension increment of 1.2 gm. (and this indicates, incidentally, that Young's modulus for this sartorius is 800 gm./cm.^{2}, which is within the range (see Fig. 2) of such resting muscle). Following these tests, the muscle was maximally tetanized for 4 sec. as indicated at the extreme left of Fig. 4 *b*. The rest of this figure then shows the effects of a series of ten 2 mm. stretch-release tests applied to the muscle during the next 2 minutes. Consider first the behavior of the muscle in the released state before each stretch. For the first four such periods, steady maintenance of the base line indicates that rigor is absent. At the fifth, there is a barely detectable, very slowly developing rigor, and then in the remaining periods its development becomes more and more evident as shown by the progressively increasing rate of tension rise. Thus these results prove that, after a rigor "latent period" of about 50 sec., there follows a phase of increasing intensity of active state like that described in the preceding section. The corresponding tensions developed in the series of stretched states show a parallel behavior, a small, apparently constant change in the first four (equal to that of the unstimulated muscle, and thus indicating that no change in rigidity occurs during the rigor latent period), and then increasingly larger increments in succession in the six that follow. The fact that each of these latter increments of tension levels off after a more or less sharp though always relatively small "overshoot," demonstrates that at each such moment there is a definite increase in rigidity of the muscle. Since each such increase was obtained by a constant stretch of the muscle at a constant length, the relative increases in rigidity are directly comparable in terms of the relative recorded deflections.
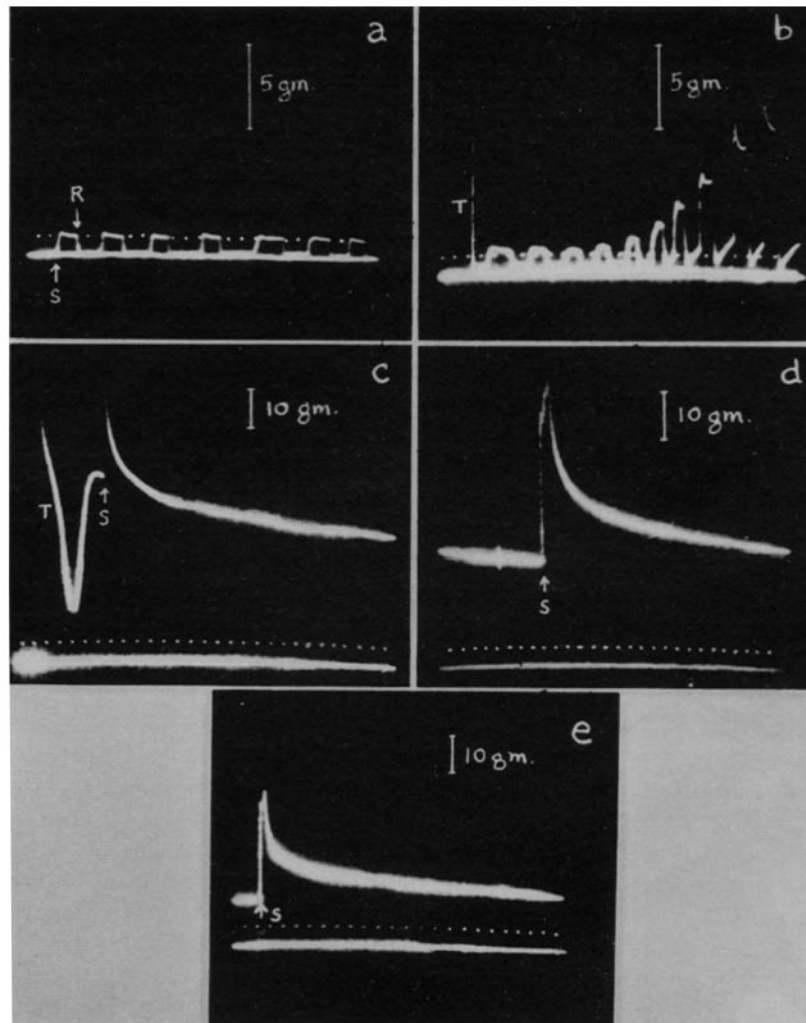
FIG. 4a and 4b. Effects of quick 2 mm. stretch and 2 mm. release on tension of IAA-poisoned muscle. (a) Muscle at rest: S indicates stretch, R, release, and subsequent similar effects were produced by repetitions of these treatments. (b) Same muscle after 4 sec. tetanus (T indicating terminal fatigue of this activity) and then subjected to a series of stretch-release cycles (as in (a)) during latent period and then development of rigor tension.

FIG. 4 c, 4 d, and 4 e. Effects of 2 mm. quick stretch (5) on tension during plateau (c) and later phases (d, e) of rigor cycles in different IAA muscles each tetanized to full fatigue for production of rigor. Time of application of stretch after start of relaxation of rigor: (c) 5 sec.; (d) 2.5 min.; (e) 4.5 min.

Tension outputs of muscles: (a) and (b) P_O = 30 gm.; (c) P_O = 80 gm., P_{OR} = 49 gm.; (d) P_O = 70 gm.; P_{OR} = 36 gm.; (e) P_O = 58 gm.; P_{OR} = 18 gm. Timing and initial base line as in Fig. 3. See text for further details.

876

And these indicate that when the active state is most intense, as judged by the rate of rise of rigor in the released state, the rigidity has increased to 8 to 9 times the value present at rest—a result like that found in the previously discussed isotonic work on elasticity. Now, as discussed in the section on elasticity variations, a measure of the changing intensity of the active state of a responding muscle is given by its alterations in rigidity. It is therefore evident from the results of Fig. 4 *b* that a muscle going into isometric rigor relatively rapidly develops its active state, and that the intensity of this state at each moment of this period is always greater than if this were judged by the corresponding actual rigor tension then present in the unstretched muscle. This behavior is in general like that found by Hill (10) for a muscle during onset of a twitch. Our results, however, do not seem to demonstrate any diminution in compliance during the rigor latent period as did Hill's for that part of the twitch; but this portion of the response is in need of further study.

Fig. 4 *c*, 4 *d*, and 4 *e* show what occurs when the stretch is applied at progressively later points in rigor. The results are in general comparable to those of Hill (10) obtained during corresponding points of a twitch (see his Fig. 6), *i.e.* the immediate, large increments of tension are not maintained but they each fall to a level near that which the muscle would have reached during an undisturbed course of its rigor changes at the new stretched length. These results show that at about plateau of rigor (Fig. 4 *c*) and thereafter (Fig. 4 *d* and 4 *e*) the muscle cannot bear the extra tension immediately set up in it by the stretch; it "gives" or "slips" to a lower level of tension. And this indicates that the active state property of the contractile component of the muscle in rigor—the capacity to bear a load—is maximum at rigor plateau (and its value is given then by the existing rigor tension), and that thereafter the intensity of this property falls in rough parallelism with the fall of rigor tension.

<div align="center">DISCUSSION</div>

Our results demonstrate that a muscle in IAA rigor possesses active state mechanisms qualitatively like those of a muscle in normal contraction. Particularly significant is the finding that on passage of a muscle into rigor, the contractile component develops the two fundamental properties of the active state: a capacity to shorten which is governed by a hyperbolic relation between force and speed, and a capacity to bear a load whose maximum is equal to the greatest rigor tension developable by the muscle. Our work deals only incidentally with the series elastic component. But the result that a small, quick release of a muscle at plateau of isometric rigor causes immediate disappearance of developed tension proves that such a component exists in the rigor muscle and that it is passive, undamped, and of low compliance. The general similarity of these properties to those of this component in the normal case suggests that the state of rigor has no effect on the series elasticity of muscle.

Our work provides only a general description of the kinetics of the rigor active state, because the kinetics and general output of a rigor response vary so greatly with the nature of the conditioning activity impressed on the poisoned muscle, and even when any one kind of such activity is used. Thus we have no rigor responses, such as the maximal twitch and tetanus of normal contraction, to serve as standards in reference to which precise comparisons can be made of the kinetics of the underlying active state. However, all rigor responses are alike in general outline for they involve an initial relatively fast phase of shortening or tension development, a more-or-less extended period of plateau, and then a quite slow and prolonged phase of relaxation. In relation to such a generalized temporal outline, our results demonstrate that the underlying active state (either in isotonic rigor, as shown, e.g., by variations in Young's modulus, or in isometric rigor as indicated by rate of redevelopment of tension following quick release and by quick stretch effects) develops more abruptly than does the directly recorded contractile change, tends to plateau for a short time, and then decays. In this general kinetic sense, the intensity of the active state of rigor has a pattern like that of the normal active state. However, the time course of the active state of rigor is more like that of a long, slow tetanus than of a twitch, since the potentiality of greatest tension output—as represented by the greatest capacity of the rigor-activated contractile component to bear a load—is attained as actually recordable tension at plateau of rigor. If the rigor response were more comparable to a twitch, its maximal tension output would be less than the full capacity to bear a load (10). We emphasize, however, that our comparison of a rigor sequence with a sort of tetanus is limited in that, once the active state of rigor has run its course, the muscle is not capable of subsequent repetition of rigor activity. This is in contrast to a tetanus, or any kind of true contraction, in which the active state processes are of such a nature as to permit recurrent cycles of contractile activity.

Although the foregoing demonstrates the qualitative similarity of the contractile component's active state properties in rigor and in normal contraction, there are quantitative differences. Mention has already been made especially of the constant, b, of the force-velocity equation, which is very much smaller (740 times) for rigor. Since the value of this constant is indicative of the rate of release of total energy associated with shortening of the contractile units, this rate in rigor must be very much less than in ordinary contraction. Another quantitative difference is indicated by the result that application of a quick stretch to a muscle during development or at plateau of its isometric rigor causes a tension output which at most—after "give" from overshoot—is equal to the value of P_{OR}. Now, under the most favorable conditions, the greatest P_{OR} is only about 0.5 to 0.6 of P_O (the maximal tetanus tension); we must therefore conclude that the contractile component in rigor develops a maximal

load-bearing capacity which is of the order of only about half of that in normal contraction.

In a normal response the contractile component develops the active state when the muscle is stimulated and there thus occurs in the fibers the sequence of events of excitation—contraction coupling (22–24); *i.e.*, membrane events are linked in a triggering action to activation of the contractile material. But, in setting up rigor, excitation is not involved, and thus the process of activating the contractile components of the IAA-poisoned muscle depends only on the mechanochemical properties of the contractile system; *i.e.*, on the reactions by which chemical sources of potential energy are made available to the contractile protein for performance of mechanical work.[4] In this respect the IAA system is like the reconstituted models of muscle that have been studied so much. But the rigor system occurs in an unextracted muscle, and the mechanochemical reactions that cause rigor development, though profoundly modified by the metabolic effects of IAA poisoning, are inherently characteristic of muscle as such.

The immediate effect of IAA is inhibition of muscle glycolysis by a direct suppression of activity of the triose dehydrogenase system. But the effect which is presumably of special significance in the mechanochemistry of rigor production is an indirect one, the irreversible dephosphorylation of ATP (adenosinetriphosphate). This reaction, and other special features of the behavior of ATP in the IAA muscle, provide the basis for a previously proposed outline of mechanochemical coupling in a rigor response (4). Be that as it may, we are at least certain that generation of the active state of rigor is definitely accompanied by ATP splitting. A number of attempts have been made recently to determine whether a similar ATP reaction occurs in association with other, *i.e.* normal, active states (for review, see Weber (25)). Some of these findings may be interpreted to indicate such an association; but certain results, especially in the work of Mommaerts (26) on the normal twitch, show no concomitant dephosphorylation of ATP. Weber assumes that the ATP appears not to breakdown because it is rapidly resynthesized "from an unknown phosphagen." If such a substance exists in muscle, then the irreversible splitting of ATP in rigor suggests that an effect of the IAA may be an inactivation of the alleged function of the phosphagen in resynthesis of ATP.

In any case, the role of ATP breakdown under IAA poisoning is not too clear, since rigor occurs in association with this reaction only after the ATP concentration has been decreased to a level lower than about 50 per cent of its

---

[4] Excitation is, of course, used as part of the conditioning activity that provokes the muscle to produce rigor, but this is only incidental: the rigor under such conditions can be made to appear altogether later than the time of the conditioning excitations; and, moreover, rigor will develop in an IAA muscle that has not been subjected to any excitation.

normal value (2). Thus irreversible splitting of ATP as such is not sufficient to set up the active state of rigor, for, if it were, this response should develop in relation to the hydrolysis of the first 50 per cent of the muscle's ATP. However, this may signify that during this part of the change the net rate of ATP breakdown is not high enough to activate a mechanical change, as seems to be the case for initiation of shortening in rigor mortis (27); or possibly a relaxation mechanism is maintained that prevents the ATP splitting from engendering the active state, as suggested by Weber (25). But still another interpretation is that in IAA rigor even the delayed breakdown of the ATP is merely coincidental with and not causally related to rigor production, and that therefore a reaction of some other substance, e.g. another nucleotide (25), serves as the primary agent in generation of the active state of the poisoned muscle. None the less, among the various nucleotides that have been studied in relation to induction of contraction in reconstituted models, ATP stands out by far as the most effective, and thus it appears to be the most probable agent in energizing physiological contraction (25).[5] In any case, it is provocative that the breakdown of ATP, which is so moot in relation to normal activity of muscle, does occur in association with development of the active state of rigor. Clarification of this contradiction, and thus elucidation of the chemical basis of normal contraction, may come from further studies of the chemical changes of the muscle in rigor and the correlation of these with associated mechanical events.

REFERENCES

1. Lundsgaard, E., *Biochem. Z.*, 1930, **217**, 162.
2. Lundsgaard, E., *Biochem. Z.*, 1930, **227**, 51.
3. Lundsgaard, E., *Biochem. Z.* 1934, **269**, 308.
4. Sandow, A., and Schneyer, C. A., *J. Cell. and Comp. Physiol.*, 1955, **45**, 131.
5. Sandow, A., and Mauriello, G. E., *Fed. Proc.*, 1955, **14**, 129.
6. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1938, **126**, 136.
7. Hill, A. V., *Proc. Roy. Soc., London, Series B*, 1950, **137**, 40.
8. Wilkie, D. R., *Brit. Med. J.*, 1956, **12**, 174.
9. Sandow, A., *Science*, 1958, **127**, 760.
10. Hill, A. V., *Proc. Roy. Soc., London, Series B*, 1949, **136**, 399.
11. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1950, **137**, 273.
12. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1953, **141**, 104.
13. Dickinson, C. J., Electrophysiological Technique, London, Offices of Electronic Engineering, 1950.
14. Mostofsky, D., and Sandow, A., *Electronics*, 1951, **24**, No. 11, 114.
15. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1951, **138**, 329.

---

[5] In this respect, it is also significant that in some preliminary work (28) we have found that muscle in rigor, first made to relax relatively quickly by treatment with sodium pyrophosphate, recontracts under action of ATP.

16. Katz, B. J., *J. Physiol.*, 1939, **96**, 45.
17. Dixon, W. J., and Massey, F. J., Jr., Introduction to Statistical Analysis, New York, McGraw-Hill Book Co., Inc., 1951.
18. Aubert, X., *Arch. Internal. Physiol.*, 1953, **61**, 116.
19. Aubert, X., Le Couplage energétique de la contraction musculaire, Brussels, Editions Arscia, 1956.
20. Gasser, H. S., and Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1924, **96**, 398.
21. Ritchie, J. M., *J. Physiol.*, 1954, **126**, 155.
22. Katz, B., *Proc. Roy. Soc. London, Series B*, 1950, **137**, 45.
23. Sandow, A., *Yale J. Biol. and Med.*, 1952, **25**, 176.
24. Csapo, A., and Suzuki, T., *J. Gen. Physiol.*, 1958, **41**, 1083.
25. Weber, H. H., *Ann. Rev. Biochem.*, 1957, **26**, 667.
26. Mommaerts, W. F. H. M., *Am. J. Physiol.*, 1955, **182**, 585.
27. Bendall, J. R., *J. Physiol.*, 1951, **114**, 71.
28. Sandow, A., and Mauriello, G. E., *Fed. Proc.*, 1954, **13**, 125.