STUDIES ON THE RIGOR RESULTING FROM THE THAWING OF FROZEN FROG SARTORIUS MUSCLE*

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INTRODUCTION

Studies of the effect of below zero temperatures on the isolated frog muscle fibre by Chambers and Hale (1) have shown that, within limits, partial freezing does not seriously affect the ability of the fibre to undergo contraction after it has been brought back to room temperature. With longer exposures and lower temperatures the ability to contract is lost and thawing then produces an irreversible rigor. Moran (2) has demonstrated that frog muscle loses its power to respond to electrical stimulation after thawing if 78 per cent or more of its water content is converted to ice, which happens when the temperature within the muscle is maintained at −2°C. for a day or two. Under conditions of extremely rapid freezing and thawing, such as can only be obtained with a bundle of a few fibres at the most, Thoennes (3) has cooled muscle down to liquid air temperatures so that it retains its property of contracting under electrical stimulation. Rapid freezing such as this, at the rate of thousands of degrees per second, ensures that the water is solidified in the vitrified form and that there is no formation of ice crystals which can damage the internal structure of the cell, as happens when muscle is slowly frozen.

If slowly frozen muscle is allowed to thaw at room temperature it goes into a very pronounced irreversible rigor which differs from the usual rigor of death in that the latter usually involves a shortening of 15 per cent (Bethe (4)), whereas the former produces a 70 per cent decrease in length. Walker (5), working in Hermann's laboratory in 1871, first reported this pronounced shortening and noticed that it was independent of the degree or duration of the cooling so long as the muscle was completely frozen. Although other workers such as Moran (2), Manigk (6), Deuticke (7), and Chambers and Hale (1) refer to this phenomenon, with the exception of a recent communication by Szent-Gyorgyi (8) little attempt has been made either to explain it or to derive from it information about the process of contraction in living muscle. Du-

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buisson (9) has suggested that the shortening which takes place on thawing "is bound to a process of dehydration which causes a splitting of the lipo-protein complexes of the muscle machine."

This shortening on thawing, or "thaw rigor" as it will be called, bears considerable resemblance to the synaeresis of actomyosin threads, both in the extent of shortening and in the loss of water which accompanies it. In contrast to the actomyosin thread which shows isodimensional shrinkage in the presence of ATP, thawed muscle (like the normal functioning tissue) shortens with an increase in cross-section.

Szent-Gyorgyi (10) has observed that the slices, obtained when muscle is washed in distilled water for several days at 0°C. and sectioned parallel to the fibres, show violent contraction in the presence of low concentrations of ATP. It is of interest to decide whether thaw rigor is the result of a similar synaeresis and to investigate its relation to the events in normal muscle.

Experimental Methods

Sartorius muscles from *Rana pipiens* were dissected out attached to a small piece of the pelvic bone to facilitate fastening the muscle onto a platinum electrode in the base of the glass muscle chamber. The distal end of the muscle was connected by a fine platinum wire to a lever after-loaded so that the actual load on the muscle was 9.5 gm., and the whole immersed in oxygenated Ringer's solution pH 7.3 (0.65 per cent NaCl, 0.01 per cent KCl, 0.02 per cent $CaCl_2$, 0.191 per cent Na_2HPO_4, 0.0184 per cent NaH_2PO_4). Inhibitors were dissolved in this medium, except in those experiments with $CuSO_4$ when unbuffered Ringer's solution was used and the pH was finally adjusted to 6.1 with a drop of very dilute alkali. Matched muscles attached to similar lever systems and identically loaded, were connected up in the same circuit so that when one of the muscles had been treated with an inhibitor its response could be compared with that of an untreated control stimulated simultaneously. Usually, after treatment with an inhibitor the chamber was rinsed out and stimulation of control and poisoned muscles was carried out with fresh Ringer's solution in both chambers. Stimulation was effected by a Harvard inductorium, the primary circuit of which included a rotary contact breaker driven by a synchronous motor. When a muscle was stimulated in Ringer's solution containing 0.25 mg. curare per ml. there was no difference in its response from that of a matched control in Ringer's solution alone, indicating that direct stimulation was being obtained.

For thaw rigor experiments, muscles which had been carefully dissected free from bone and tendon were placed on a glass microscope slide in a Petri dish containing sufficient Ringer's solution to maintain an atmosphere saturated with water vapour, but not enough to cover the slide and immerse the muscle. Freezing at −22°C. and thawing at room temperature were carried out in the Petri dishes to ensure that there was no loss in weight due to evaporation. Muscles were left for 30 minutes after removal from the cold room before any measurements were made. For recording kymographically the length changes on thaw, the muscle attached to the lever system, appropriately loaded, was frozen mounted in the chamber from which the Ringer's solution had been drained.

ATPase Activity.—The enzymic activity of myosin was determined by the method of Bailey (11). In addition to myosin, each tube contained 1.0 ml. 0.2 M glycine buffer pH 8.9, 0.1 ml. 0.1 M $CaCl_2$, 0.25 ml. ATP solution (containing 0.33 mg. 10 minutes P), and the total volume was made up to 1.95 ml. with 0.5 M KCl. After incubation for 10 minutes at 37°C. the reaction was stopped with 1.5 ml. 10 per cent trichloracetic acid. For each sample of myosin, three determinations each with different amounts of the enzyme were carried out. The initial rate of splitting, used to calculate the Q_p, was deduced from the straight line graph obtained by plotting phosphorus liberated from ATP against the amount of myosin used. Myosin concentration was taken as six times the nitrogen of the enzyme solution as determined by the micro-Kjeldahl method.

Myosin.—Myosin was prepared after Bailey (11) by homogenizing two sartorius muscles in 3 ml. of a solution containing 0.5 M KCl, 0.03 M $NaHCO_3$ with a Dounce type homogenizer. It was precipitated twice only because on continued precipitation frog myosin tends to become insoluble, although it retains enzymic activity.

ATP.—ATP was prepared from rabbit muscle by a method similar to that of Needham (12), and finally purified by precipitation of the Ba salt in 50 per cent ethanol at pH 1.

The author is indebted to the kindness of Dr. J. N. Stannard for gifts of iodoacetic acid and *p*-chloromercuribenzoate.

RESULTS

When a normal resting muscle is cooled to temperatures below 0°C., it undergoes slight spontaneous contraction (Dubuisson (9)), so that usually when it freezes its length is less than the original resting value. A muscle which is no longer excitable due to exhaustion or other reasons does not show this contraction, and in the data presented shortening is expressed in terms of the resting length whenever this is known. If frozen frog sartorius muscle is taken out of the cold room at −22°C. and allowed to stand at room temperature, shortening takes place as soon as thawing is complete. In some cases, for example when left on a glass surface moistened with Ringer's solution so that shortening can take place freely, the decrease in length may take place irregularly and cause the muscle to curl up. Usually the rigor is complete within 10 to 15 minutes of removing the muscle from the cold to a room temperature of approximately 20°C., but most of the shortening takes place within 2 to 3 minutes once thawing is complete. Finally the muscle is about 30 per cent of its original resting length.

The effect is essentially the same in all normal muscles whether they are frozen in the resting condition, or immediately after electrical stimulation to exhaustion, or fixed during isometric tetanus by immersion in an acetone-solid CO_2 mixture (Table I).

Although the length of time the muscle is kept frozen has no effect on the final state, there is some indication that those muscles kept at −22°C. for 90

minutes take a little longer to reach the final shortened length compared with matched muscles kept frozen for 18 hours. If muscle is rapidly frozen by plunging into an acetone-solid CO_2 mixture it is found that 30 seconds is quite adequate to produce thaw rigor.

TABLE I

The Shortening of Unpoisoned Frog Sartorius Muscle after Thawing

Experiment No.	Conditions	Muscle	Original length	Frozen length	Thawed length	Shortening
			mm.	*mm.*	*mm.*	*per cent*
1	Overnight −22°C.		31	31	10	67
2	Overnight −22°C.	R.	25	23	10	60
		L.	29	26	9	69
3	Overnight −22°C.	R.	29		9	69
		L.	29		10	66
4	Frozen in acetone -CO_2 1 min.	R. resting		22	9	59
		L. exhausted 36 stimulations per min.		25	9	64
5	Frozen in acetone -CO_2 1 min. during isometric tetanus	R.		29	9	69
		L.		27	8	70
6	Frozen in acetone -CO_2 during tetanus of freely suspended muscle	R.	36	14	9	75
		L.	33	12	7	79
7	Left 3 days in Ringer's solution at room temperature until no longer excitable before storing overnight at −22°C.		33	33	33	Nil
8	6 days in Ringer's solution at 5°C. until no longer excitable. Overnight at −22°C.	R.	22	22	22	Nil
		L.	26	26	26	Nil

The shortened muscle no longer respires; in appearance it is turgid, thicker, and resting in a pool of exuded fluid. Muscles stored at room temperature or

at 5°C. until they are no longer excitable fail to show the shortening which accompanies thaw rigor.

The loss of water or synaeresis and shortening are effects not shown by other tissues under comparable conditions. A very similar phenomenon, the "contraction" of actomyosin threads, is accompanied by an increase in protein concentration from 2 to 3 per cent to 50 per cent; *i.e.*, a 95 per cent decrease in volume and a similar loss in weight. Whole sartorius loses 35 per cent of its weight during thaw rigor if it is frozen in the resting condition; and 25 per cent if it is frozen while the unloaded muscle is tetanized to maximum shortening (Table II).

Under the latter conditions the already considerably contracted muscle does shorten a little more on thawing out, and the loss of water indicates that al-

TABLE II

Weight and Length Changes in Thaw Rigor

State of muscle when frozen	Resting	Iodoacetate-poisoned and exhausted, or kept in N_2 at fixed length	Tetanized to maximum shortening
No. of experiments	6	10	9
Loss in weight, *per cent*	34.6	−0.1	25.2
Length of frozen muscle, *mm*	29.7	29.5	12.0
Length after thawing, *mm*	8.9	26.9	9.4
Decrease in length, *per cent*	69.9	8.6	21.4

Muscles were treated for 60 minutes at room temperature, approximately 20°C., with 0.00032 M iodoacetic acid in Ringer's solution.

though the muscle has been fixed in the contracted state, the actomyosin is still capable of undergoing synaeresis.

Fig. 1, taken from kymograph records, represents the time relations of thaw rigor and indicates that although most of the shortening takes place rapidly, soon after the muscle has thawed, a less extensive, slower phase appears to follow. There is some suggestion that this biphasic effect is more apparent when muscle is frozen for 90 minutes or less. Occasionally a not very pronounced flat peak is observed in the traces. Szent-Gyorgyi has reported, since the work described here was completed, a double peak in the tension-time curve of a thawed frog sartorius. It is possible that the flat peak in Fig. 1 indicates a similar effect which is less marked due to the slower rate of thaw in these experiments, but it could also be due to a slight curling of the muscle as a result of irregular shortening, so that when the curl disappears at the end of the rapid shortening phase, the straightening out of the muscle is recorded on the trace as an apparent lengthening.

When muscle is allowed to thaw working against the very slight resistance

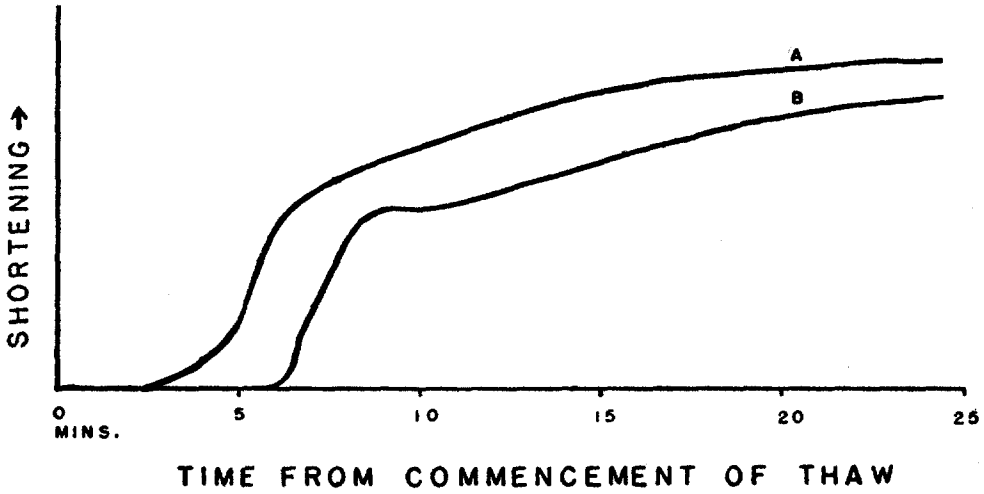


FIG. 1. Shortening of resting, matched frog sartorius muscles frozen at −22°C. and allowed to thaw at room temperature, 20°C. A, right muscle frozen for 16 hours. B, left muscle frozen for 90 minutes.

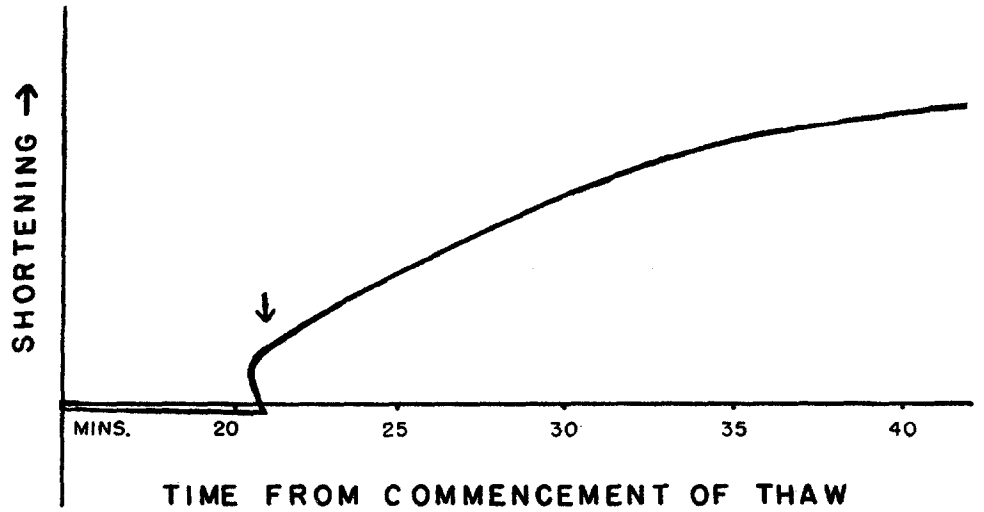


FIG. 2. Length changes of frog sartorius frozen in the resting condition and allowed to thaw with a load of 8 gm. At the time indicated by the arrow the load was removed.

of a light, unloaded heart lever, the shortening curves resemble those obtained by Buchtal *et al.* (13) with actomyosin threads in the presence of ATP. Shortening still takes place under a load of 0.8 gm., but the maximum rate of shortening

is less than that obtained with unloaded muscle. A load of 8 gm. is sufficient to reduce the shortening by 80 to 100 per cent (see Fig. 2). If shortening is prevented in this manner and the load is removed after a period adequate to allow completion of thaw rigor in unloaded muscle, the muscle immediately commences to shorten and finally approaches the length to be expected if there had been no loading.

Effect of Inhibitors on Thaw Rigor.—

Frog sartorius muscle shows changes in the mechanical response to direct electrical stimulation when it is treated with such inhibitors as (1) azide and cyanide which are effective in the oxidative enzyme systems (Stannard (14)); (2) 2:4-dinitrophenol presumably preventing phosphorylation coupled to oxidation (Hotchkiss (15, Cross *et al.* (16)); (3) sulfhydryl reagents such as iodoacetate, hydrogen peroxide, *p*-chloromercuribenzoate, and copper. These changes are briefly summarized in Table III.

With the exception of the experiments in which hydrogen peroxide and iodoacetate are used, no significant inhibition of the shortening of thaw rigor is observed, even if the poisoned muscles are frozen in the exhausted rather than in the resting state. Muscles frozen after being rendered inexcitable to direct electrical stimulation by higher concentrations of azide, copper, and cyanide still go into thaw rigor. Some inhibition is observed in muscle which has become inexcitable after 120 minutes at 18°C. in contact with 0.029 M hydrogen peroxide.

If muscle is immersed for 60 minutes in Ringer's solution containing 0.00032 M iodoacetic acid and then frozen in the resting condition, on thawing it shortens almost to the same extent as the unpoisoned control; but if the poisoned muscle is exhausted before freezing, very little shortening takes place (Table IV). The kymograph traces represented in Figs. 3, 4, and 5 demonstrate this; and they also indicate that, once the rapid shortening of thaw is over, in the resting iodoacetate-poisoned muscle there is no slower prolonged shortening such as is obtained with normal muscle.

The failure of the exhausted muscle to shorten cannot be explained by an inactivation or degradation of the myosin caused by electrical stimulation of the poisoned muscle. If ATPase activity is taken as a criterion of the functional condition of myosin (and there is evidence to show that enzymic function depends very closely on the integrity of the myosin molecule: Engelhardt (17); Bailey and Perry (20)), no change in the myosin can be demonstrated. In one experiment, for example, the Q_p's of myosin prepared from matched groups of poisoned muscles in the resting and in the exhausted condition were 390 and 393 respectively.

Thaw rigor can also be prevented by taking advantage of the observations of Lundsgaard (18, 19) which showed that when a muscle is kept in nitrogen

TABLE III

Effect of Inhibitors on Mechanical Response and Thaw Rigor

Inhibitor	Serial	Concentration M × 10^4	Time in contact	Temperature	Mechanical response	Condition on freezing	Shortening
			min.	°C			*per cent*
p-chloromercuribenzoate		3	60	18	Only slightly impaired initially but falls off rapidly	R. resting L. exhausted	68 60
Hydrogen peroxide	25/3/A	290	60	18	Falls off rapidly	R. resting L. exhausted	60 62
	25/3/B	290	120	18	Not excitable	R. L.	17 27
Sodium cyanide	26/3	18	60	17.5	Slightly impaired	R. resting L. stimulated	64 63
	28/3	200	73	19.5	Not excitable	R. L. unpoisoned control	68 68
Sodium azide	24/3/A	100	60	16.5	Not excitable	R. L.	69 72
	24/3/B	50	60	17	$\frac{1}{3}$ of control	R. stimulated L. unpoisoned control	65 71
	23/3	20	66	18	Very slightly impaired	R. stimulated L. resting	71 76
2:4-Dinitrophenol	29/3/A	10	60	20.5	Initial response similar to control but falls off rapidly	Exhausted	71
	29/3/B	10	60	23	" "	Exhausted	52
Copper sulfate	11/2	5	60	17	$\frac{2}{3}$ control, falls off fairly fast	R. stimulated L. unpoisoned control	67 69
	8/2	10	60	19	Not excitable	R. L. unpoisoned control	65 64

Muscles were stimulated for 0.6 second every 10 seconds and the amount of contraction, as represented by the kymograph record, was compared after poisoning with the response during a control period of stimulation before poisoning. Experiments with $CuSO_4$ were carried out in unbuffered Ringer's solution at pH 6.1.

after iodoacetate poisoning the phosphocreatine and ATP slowly disappear. At the same time, if it is freely suspended, the muscle goes into rigor and

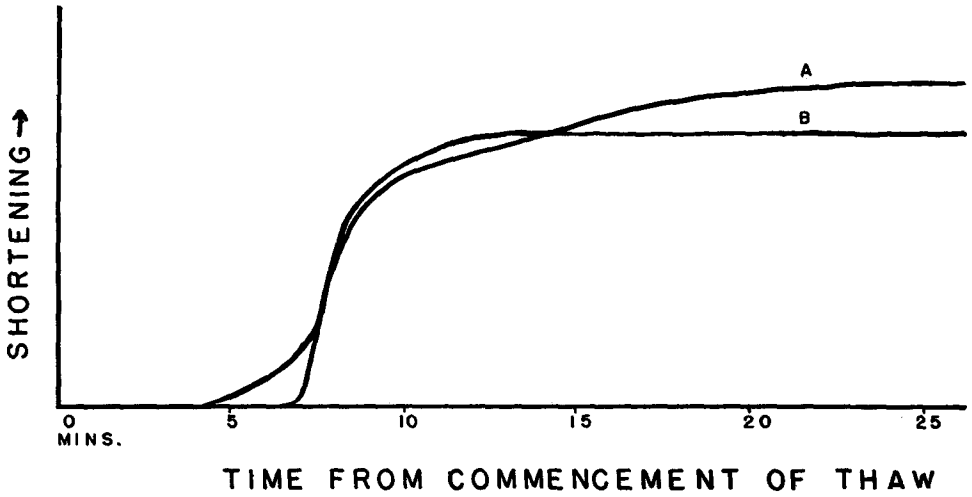


FIG. 3. Effect of iodoacetic acid on the shortening accompanying thaw after freezing for 15 hours at −22°C. A, right muscle, resting unpoisoned. B, left muscle, 60 minutes at 18°C. in 0.00032 M iodoacetic acid and frozen in the resting condition.

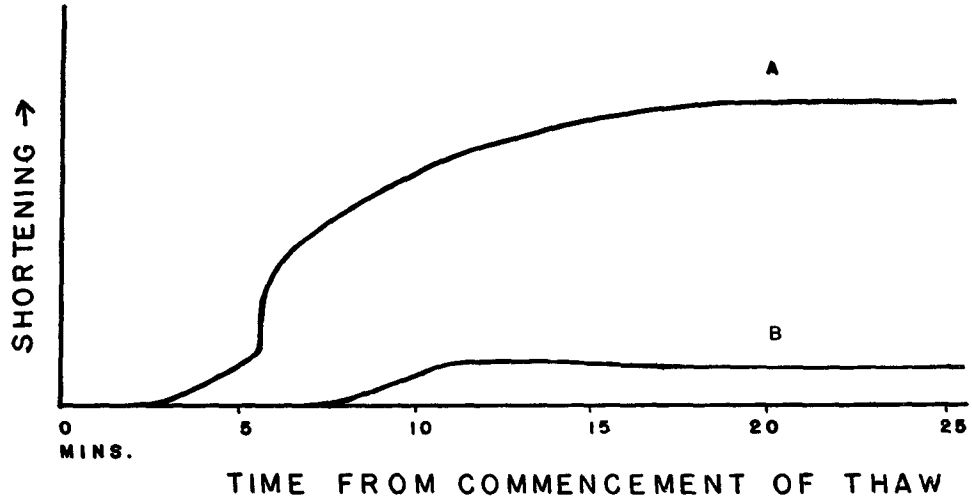


FIG. 4. Effect of exhaustion on the shortening accompanying thaw of iodoacetate-poisoned frog sartorius. Frozen 17 hours at −22°C. A, right muscle, resting unpoisoned. B, left muscle, 60 minutes at 20°C. in 0.00032 M iodoacetic acid and exhausted.

shortens, finally reaching about one-third of its resting length in a few hours. If, however, the muscle is maintained fixed at its resting length, after about

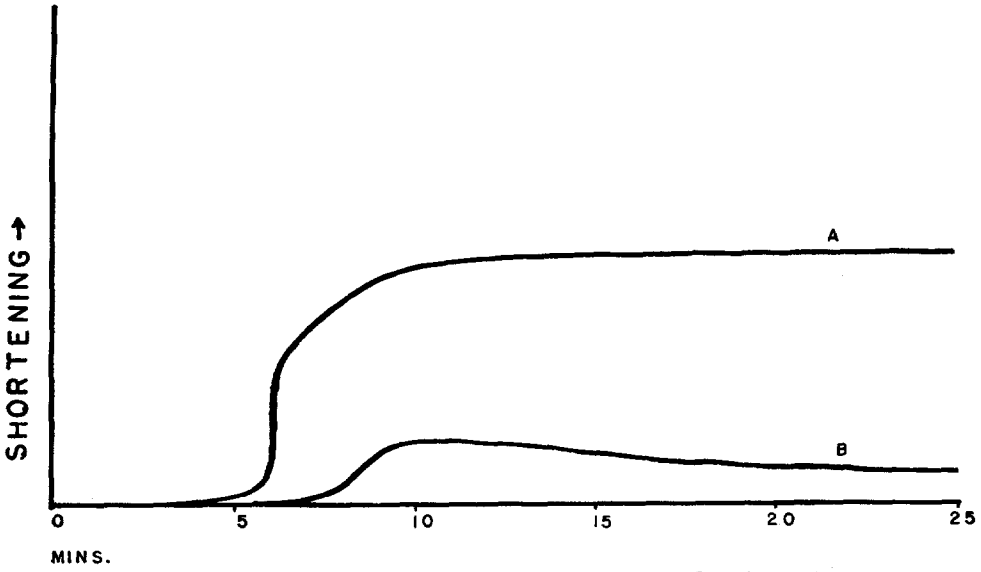


FIG. 5. Effect of exhaustion on the shortening accompanying thaw of iodoacetic acid-poisoned frog sartorius. Both muscles were treated for 60 minutes at 17°C. with 0.00032 M iodoacetic acid. Frozen 20 hours at −22°C. A, right muscle resting. B, left muscle, exhausted.

HOURS IN 0.0003M p-CMB	SHORTENING ON THAW (%)	MYOSIN ATPase ACTIVITY (Q$_r$)	NO p-CMB	p-CMB
0	72	923		
1	67	942		
3	60	826		
5	57	899		

FIG. 6. Shortening of thaw rigor, and ATPase activity of myosin extracted from frog sartorius treated with *p*-chloromercuribenzoate.

5 to 6 hours in nitrogen it loses its excitability and becomes stiff in texture, in strong contrast to the resting tissue. When such a muscle is frozen and subsequently thawed it shortens very little or not at all, although myosin prepared from such a muscle shows the normal ATPase activity. As in the case of exhausted iodoacetate-poisoned muscle, the slight amount of shortening which sometimes occurs on thawing is presumably an index of how well the ATP has been removed.

Iodoacetate-poisoned muscles, which owing to prior stimulation or to keeping in nitrogen at fixed length show little or no shortening, lose no weight on thaw-

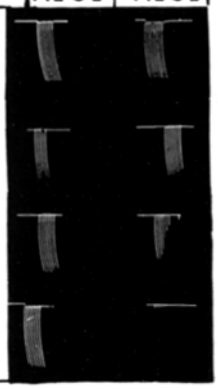
HOURS IN 0.029M H_2O_2	SHORTENING ON THAW (%)	MYOSIN ATPase ACTIVITY Q_P	NO H_2O_2	H_2O_2
0	58	644		
1	57	327		
2	48	578		
4	27	538		

FIG. 7. Shortening of thaw rigor, and ATPase activity of myosin extracted from frog sartorius treated with hydrogen peroxide.

ing (Table II). This indicates that the loss of water is not a result of freezing and thawing *per se*, but is an effect accompanying shortening.

In vitro, low concentrations of *p*-chloromercuribenzoate and hydrogen peroxide readily inhibit the ATPase and actomyosin-forming activities of myosin in a manner which suggests that the two properties are closely related (Bailey and Perry (20)). The enzymic function of myosin in the presence of these inhibitors can thus be taken as a good index of its actomyosin-forming activities. Myosin extracted from whole sartorius muscle which is treated with *p*-chloromercuribenzoate for periods of 1 to 5 hours shows little change in ATPase activity; nor is there much change in the extent of thaw rigor which takes place. After 1 hour in contact with the poison, muscle shows 20 to 30 per cent decrease in oxygen uptake during recovery from electrical stimulation. With the longer periods of exposure mechanical response falls off rapidly, until after 5 hours it is no longer evident (Fig. 6). This inability to contract on direct

electrical stimulation cannot be attributed to inactivation of the ATP-splitting function of myosin.

With hydrogen peroxide there is a slight falling off of ATPase activity, paralleled by a more pronounced decrease in the extent of shortening on thawing, although the mechanical response does not appear to be so sensitive to this substance as it is to *p*-chloromercuribenzoate (Fig. 7). Although the absolute

TABLE IV

Effect of ATP on Muscles Treated to Prevent the Shortening of Thaw Rigor

Serial	Treatment	Original length	Frozen length	Thawed length	Shortening (of original)	Length of thawed muscle in ATP
		mm.	*mm.*	*mm.*	*per cent*	*mm.*
	R. resting	25	23	12	52	
	L. exhausted	26	25	22	12	
	R. resting		30	15	50	
	L. exhausted		30	24	20	
	R. resting	28	22	13	54	
	L. exhausted	28	28	25	11	
28/2/A	R. resting	28	25	13	54	13
	L. exhausted	29	27	21	27	15
28/3/B	R. isometric rigor in N_2	26	26	26	Nil	15
	L. " " " "	26	25	25	4	16
22/2/D	R. resting	30	21	13	57	
	L. exhausted	30	29	26	13	15
24/2	Isometric rigor in N_2	35	35	35	Nil	20
10/5	R. exhausted	33	32	31	6	18
	L. exhausted	34	33	32	6	17

All muscles were kept for 60 minutes at 20°C. in Ringer's solution containing 0.00032 M iodoacetic acid before they were treated as indicated in table. Shortening was produced with 0.003 M ATP solution containing 0.04 M KCl and 0.0004 M $MgCl_2$, with the exception of experiment 28/2/A when the ATP was in Ringer's solution.

yields of myosin from muscle treated at the various levels with these inhibitors were not measured, no obvious variation was apparent.

Effect of ATP.—

By immersion in ATP solution shortening can be induced in iodoacetate-poisoned muscles which either have been exhausted, or left at fixed length in

nitrogen to prevent the contraction of iodoacetate rigor. Some effect is obtained on the thawed unshortened muscle with Ringer's solution containing 0.003 M ATP, but a medium similar to that used by Szent-Gyorgyi (10) to bring about the synaeresis of actomyosin threads (0.003 M ATP, 0.04 M KCl, 0.0004 M $MgCl_2$) is more satisfactory. Using this solution considerable shortening takes place, with few exceptions; the length of exhausted iodoacetate-poisoned muscle treated with ATP approached that of the control, an iodoacetate-poisoned matched muscle frozen in the resting state (Table IV). Shortening is usually complete in 15 minutes and in many cases the muscles curl up into tight balls. No shortening is obtained with Ringer's or with the KCl-$MgCl_2$ solutions alone.

DISCUSSION

Lundsgaard's classical work has indicated that in iodoacetate-poisoned muscle energy for contraction no longer can be provided by glycolysis but must come from the breakdown of phosphocreatine and ATP stores within the muscle. Stimulation of a poisoned muscle until it will no longer contract results in the depletion of these substances. Hence the absence of thaw rigor under these circumstances suggests that either phosphocreatine or ATP, more likely the latter by analogy with the actomyosin model, is necessary for the shortening to take place. Such an hypothesis is strengthened by the fact that ATP can bring about shortening in muscles in which it has been prevented by procedures that reduce the ATP to a low level. The most satisfactory explanation is that the shortening which takes place in thaw rigor is an *in situ* example of actomyosin synaeresis. Moran's results, which demonstrate that the shortening of thaw rigor can be prevented by a very prolonged thawing at temperatures just below 0° C., can be accounted for by the breakdown of ATP which takes place in such partially frozen muscle. Thus when the thaw is complete there is no longer sufficient ATP to synaerese the actomyosin and produce shortening.

In considering this effect in relation to the contraction of normal muscle it should be emphasized that thawed muscles are not excitable, that they no longer respire, and that the freezing causes considerable disorganization in the protoplasm (Chambers and Hale, (1); and Thoennes (3)). This damage is sufficient to upset the normal localization of cell constituents so that ATP, which must be in some way localized or rendered ineffective to actomyosin in the resting cell, can now interact with the myofibrils and cause them to shorten, as Schick and Hass (21) have recently demonstrated with preparations of fibrils prepared from rabbit skeletal muscle.

Whether this is the normal process in the contraction of muscle is a question which cannot yet be fully answered. The fact that aggregation and synaeresis of actomyosin can take place in a certain ionic environment even though the constituent proteins are randomly oriented with respect to one another, as in actomyosin threads, does not necessarily mean that this is the normal contrac-

tile process. More likely the synaeresis and shortening is a gross counterpart of the real process which involves a rather more delicately geared interplay of myosin, actin, ATP, and numerous other cell constituents.

It is possible that under the conditions for synaeresis of extracted actomyosin the protein complex shows maximal tendency to aggregate, a property which depends on the bonding possibilities of the chemical groups in the protein molecules; groups which have a more subtle role to play in the myofibril where electron microscope and x-ray investigations show that there is both molecular and atomic orientation of structure. Such a role must permit the contractile process to change to one of relaxation, and to produce an increase in the tensile strength of the contractile units during shortening; neither of these properties is exhibited by actomyosin threads.

There is a suggestion from the results in Table II, showing weight losses on thaw, that even in muscle which has shortened maximally under the influence of a tetanizing current the actomyosin is not synaeresed but can be made to synaerese by thawing the frozen tetanized muscle.

It is striking that myosin retains its ATPase activity so effectively even after the prolonged action on whole muscle of such powerful inhibitors as hydrogen peroxide and *p*-chloromercuribenzoate. The results suggest that either these inhibitors act at or near the muscle cell membrane; or that if they do penetrate into the cell the active groups of the myosin must be protected in some way, to prevent the inactivation which these reagents would be expected to produce if they were allowed to react freely with the protein of the myofibrils.

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SUMMARY

1. The rigor which takes place when completely frozen frog sartorius muscle is thawed ("thaw rigor"), is accompanied by a decrease in length of 70 per cent and a loss in weight of 35 per cent, whether the muscle is frozen in the resting or the exhausted condition, or during isometric tetanus. Muscle tetanized to maximal shortening shows a loss in weight of 25 per cent on thawing.

2. A load of 8 gm. is sufficient to prevent the decrease in length on thawing, but after its removal the muscle will shorten almost to the normal extent.

3. Inhibitors such as azide, cyanide, 2:4 dinitrophenol, *p*-chloromercuribenzoate, Cu, and hydrogen peroxide, when used for periods not exceeding 1 hour, have little effect on the shortening; although in some cases these poisons render the muscle inexcitable.

4. Muscles poisoned with iodoacetic acid and stimulated to exhaustion, or

maintained at fixed length in nitrogen, show little or no shortening on thawing. ATP can produce shortening in the muscles in which it has been prevented.

5. The phenomenon is considered to be due to an *in situ* synaeresis of the actomyosin of the myofibrils. As a result of the disorganisation of the muscle protoplasm produced by the freezing and subsequent thawing, the ATP, which must be bound or localized in the resting muscle, can act on the myofibril in a similar manner to its *in vitro* effect on the actomyosin thread.

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