CONTRACTILE PROPERTIES OF COMPRESSED MONOLAYERS OF ACTOMYOSIN

BY TERU HAYASHI

(*From the Department of Zoology, Columbia University, New York*)

PLATE 2

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I

INTRODUCTION

The earlier studies of the surface-spread pepsin-albumin system (1) which were continued in this laboratory (2) demonstrated that enzymes in such a physical state retained some aspects of their biological properties. Similar results were obtained by Kaplan with the intracellular enzyme catalase (3) and led to the speculation that surface-spread proteins might exist within the cell at heterophasic interfaces to form "cytostructures" (4). The studies were, therefore, extended to still another intracellular material, the muscle protein actomyosin (5).

The complex protein actomyosin is generally acknowledged to be the contractile element of muscle and is also considered to be associated with certain structural elements (6, 7). In addition, this protein has the distinctive biological activities of splitting ATP (8, 9) and of contracting under the influence of ATP (10). The fact that surface-spread myosin is reactive with ATP (11) along with the other above information pointed to the suitability of the study of the biological properties of surface-spread actomyosin.

These studies have shown that fibers of surface-spread actomyosin are capable of contracting under the influence of ATP and of lifting appreciable loads. In other words, they are capable of the basic function of muscle, that of transforming chemical energy into mechanical work.

II

Materials

The ATP used in these experiments was the commercial product obtained from the Schwartz Laboratories. A weighed sample of the free acid is dissolved in a small amount of 0.05 M KCl, and the solution brought to pH 7.0 with the addition of KOH. (All pH values are determined with the glass electrode.) The neutral solution is now buffered with veronal buffer at pH 7.6 (optimal pH determined experimentally), and brought up to volume with 0.05 M KCl solution to make the final concentration of

0.3 per cent ATP. This solution of ATP was used throughout the experiments except where otherwise specified.

Other chemical reagents used were commercial chemically pure grade reagents. Pyrex-distilled water was used throughout all experiments.[1]

The actomyosin was prepared according to the method of Szent-Györgyi (16), but with some modifications and details supplied by Dr. A. G. Szent-Györgyi in correspondence. Some details of preparation not given in the above reference are supplied below.

The overnight extract of minced muscle in Weber-Edsall (12) solution is centrifuged at 2000 R.P.M. for 15 to 20 minutes to remove the coarse detritus, the supernatant being decanted and filtered through cheese-cloth. To obtain as clear an extract as possible, the process is repeated with high speed centrifugation. To the final filtrate is now added 10 volumes of distilled water, stirred thoroughly, and the precipitate is allowed to settle overnight.

The supernatant is now drawn off and discarded, and the precipitated actomyosin (myosin B) is packed by high speed centrifigation. To the volume of packed precipitate enough solid KCl is added to give a final KCl concentration of 0.6 M KCl. The material is stirred gently until a concentrated solution of actomyosin is formed (it may be necessary to dilute slightly with 0.6 M KCl) and allowed to stand 2 to 3 hours. For preservation, an equal volume of glycerol is added to this solution of actomyosin, gently stirred, and the mixture then placed in the deep-freeze at −10 to −12°C. The activity is kept for weeks.

III

Methods

1. Formation of Fibers.—The technique of compressing protein monolayers to form insoluble fibers has been described in earlier work (2). For the current investigation, certain differences must be described, however. The Langmuir trough used was made of plexiglass, being 80 cm. long, 10 cm. wide, and 1.6 cm. deep, and the edges of the trough being covered with a smooth coat of ceresin. The movable barriers were brass strips 25 cm. by 0.6 cm. by 0.6 cm., covered with ceresin. The trough was filled with 0.05 M KCl buffered at pH 7.0.

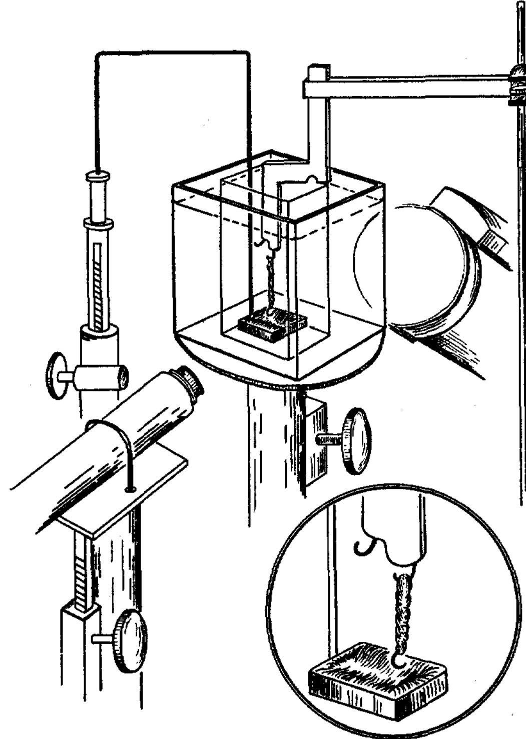
The 50 per cent glycerine actomyosin solution is diluted with 1.0 M KCl to 2 or 3 times the original volume, depending on the original concentration of actomyosin. The diluted actomyosin is now placed dropwise at the air-water interface of the trough between movable barriers to form a surface-spread film.[2]

[1] In the earlier experiments and preliminary observations (5) somewhat different solutions were used. Thus, for example, McIlvaine's phosphate-citrate buffers were employed in the earlier experiments, whereas veronal buffers were used in the later experiments, in order to make comparisons with ATP-ase determinations. The results did not differ essentially with these changes.

[2] Better spreading and larger fibers are obtained if the solution is spread from the edge of a glass slide, and such a technique was used in experiments to be published shortly. In the present work, however, dropwise spreading was employed throughout. Likewise, as found in later stages of this investigation, allowing 5 minutes for spreading resulted in more homogeneous, stronger fibers.

After 1 to 2 minutes, the film may be compressed. Especially in the last stages, the barriers should be moved slowly to minimize the trapping of air bubbles in the collapsed film. The fiber so formed may then be freed from the barriers with dissecting needles and floated at the surface of the trough for further manipulations.

2. *Determination of Water Content.*—Large fibers are placed in a moist chamber on a plastic plate (12) to minimize sticking with the fiber in edgewise contact with filter paper to draw off excess water. After 15 minutes in the moist chamber, the fiber is



TEXT-FIG. 1. Apparatus for measuring loaded contractions. Description in text.

placed in a weighing bottle and the wet weight determined. The fiber is then dried to constant weight in an oven at 100°C. and the dry weight determined. The water content of the uncontracted fiber is thus found.

For the contracted fibers, the material is first treated in the ATP solution while on the plate, to bring about an unloaded contraction. Length of the fiber is checked before and after treatment with the ATP. The contracted fibers are then treated in the same way.

3. *Determination of ATP-ase Activity.*—Several large fibers are made, and immersed in 20 ml. of the ATP solution. Half-milliliter aliquots are now removed at intervals, and the inorganic phosphate determined according to the method of Fiske and SubbaRow (13). Since this results in a constant decrease in the volume of solution relative to the fibers (enzyme) from 20 ml. to 17.5 ml., certain errors are in-

herent. Since the volume of solution as compared to the volume of fibers was so large that this error was small, corrections were not made.

4. *Loaded Contractions.*—The apparatus used is shown in Text-fig. 1. The outer chamber accommodates a stream of water from a constant temperature bath so that the temperature is maintained at 28°C. The inner chamber is the reaction vessel containing the solution being tested, the whole being mounted on a platform movable up and down with a rack and pinion.

Above the reaction vessel is a clamp which is used to hold the hook-frame supporting the fiber, so that the frame and hook will extend down into the reaction vessel when the reaction vessel is racked up. Also extending down into the reaction vessel is a supporting platform, independently movable with a rack and pinion. The entire apparatus is illuminated with a suitable light, and observations made through the telescope of a cathetometer capable of detecting movements of 0.05 mm. using a vernier scale.

To affix the fiber in the apparatus, the fiber is first formed and left floating in the trough. One end of the fiber is then tied to a suitable glass weight (density = 2.5) and the other end is tied to the lower of the two hooks on the frame. The weight at the other end of the fiber is then hung on the upper hook so that the fiber hangs slack between the two hooks. The frame is now carried to the apparatus and clamped in place above the reaction vessel. The platform is raised to a point below the hook so that the weight may be removed from the upper hook and placed on the platform in such a way that no tension is imposed on the fiber while in air. The reaction vessel containing the neutral control solution (0.05 M KCl) is now raised so as to immerse weight and fiber completely. The entire operation is carried out quickly to prevent drying of the fiber. The platform is now lowered until the weight hangs free, and the resting initial length measured. The platform is then raised again to support the weight.

To change solutions, the reaction vessel is lowered, the KCl solution drawn off quickly with a suction device, and replaced with the ATP solution. The reaction vessel is now raised quickly until the fiber is immersed; at the same time the platform is lowered so that the weight and fiber hang free, and time and length measurements begun. The contractions are therefore essentially isotonic, after-loaded contractions

IV

EXPERIMENTS AND RESULTS

(*a*) *Unloaded Contractions.*—The uncontracted actomyosin fiber presents an appearance typically like that of other protein fibers formed in this manner (1). Fig. 1 *a* shows an uncontracted fiber (low power with phase contrast) and points up the longitudinal organization of the fiber resulting from the lateral compression of the film. The fiber is essentially a rolled-up or accordion-pleated two dimensional film. Under crossed Nicols it shows a strong birefringence positive with respect to the long axis, indicating orientation of the component molecules. Under ordinary light the visible organization is in striking contrast to the precipitated fibers of Weber (14) and Szent-Györgyi (12) which lack any sign of such organization. Under high power the fibers show parallel fibril-like strands, which may be folded edges of the original film. Fig.

1 *a* also shows three air bubbles in the fiber, which have been purposely included to contrast with the same fiber in the contracted condition.

Fig. 1 *b* shows the same fiber after 2 minutes in 0.3 per cent ATP solution. In such an unloaded contraction, the fiber contracts 45 to 50 per cent, the contraction being indicated in the plate by the moving together of the three air bubbles and the change in shape of the air bubbles. It may be seen that the contracted fiber is more opaque, and shows a general change in appearance

TABLE I

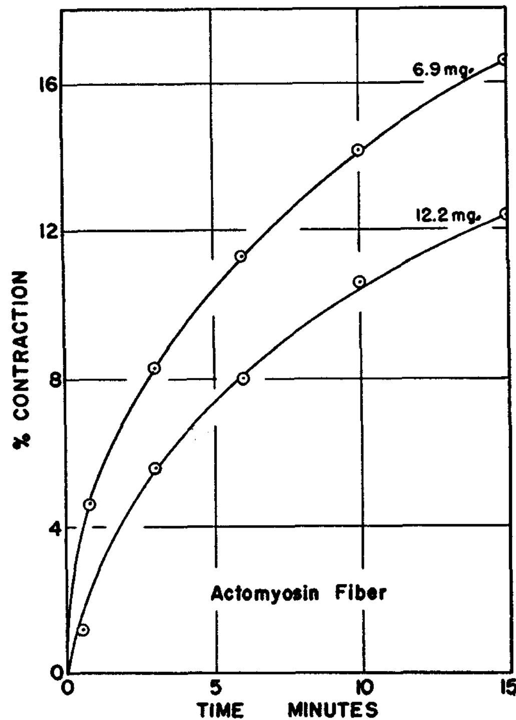
Wet and Dry Weights of Actomyosin Fibers

Sample	Wet weight	Dry weight	H_2O content
	mg.	*mg.*	*per cent*
Uncontracted			
1	72.9	3.6	95.2
2	52.4	3.1	94.1
3	40.4	2.9	93.0
4	43.0	3.3	92.3
5	23.0	2.2	93.0
Average			93.5 ± 1.0
Contracted			
1	17.8	1.0	94.6
2	40.9	3.7	90.1
3	32.2	3.6	89.9
4	21.5	1.9	91.2
5	21.2	2.0	90.6
6	17.3	1.9	89.0
7	31.5	2.5	92.1
8	26.9	2.4	91.1
9	20.2	1.7	92.3
Average			91.2 ± 1.6

best described as becoming "glassy." The diameter does not decrease markedly. Under high power observation, the parallel arrangement of the fine fibril-like strands becomes distorted, with each strand seemingly becoming more dense and "kinky." A more detailed examination of these phenomena is planned.

For these unloaded contractions, it was found that maximally contracting fibers could be formed with the trough buffered at pH 7.0 or higher. Fibers formed at pH values below 7.0 fell off rapidly in their activity, so that at pH 4.0 practically no contraction was observable. The optimal pH of the ATP-contracting solution was found to be pH 7.4–7.6.

The water content of the uncontracted and contracted fibers may be seen



TEXT-FIG. 2. Loaded contractions of actomyosin fibers. Loads 6.9 and 12.2 mg. in water (11.5 and 20.3 mg., respectively in air). Trough: 0.05 M KCl buffered at pH 7.0; 0.3 per cent ATP in 0.05 M KCl buffered at pH 7.6 veronal buffer.

TABLE II

ATP-Ase Activity of Fibers of Surface-Spread Actomyosin

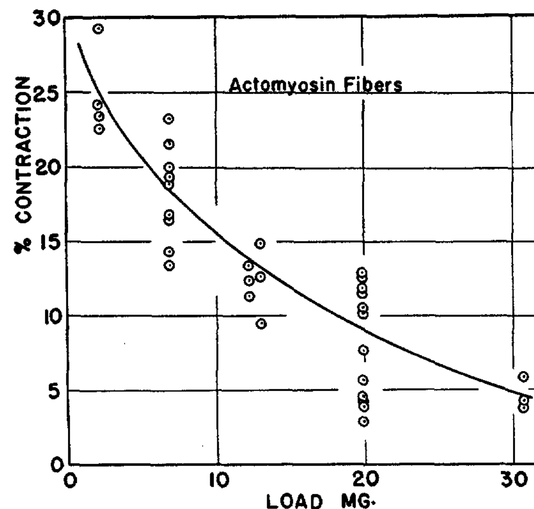
Time	$\frac{\mu g.\ P}{0.5\ ml.}$	$\frac{\Delta\ \mu g.\ P}{0.5\ ml.}$	Total $\Delta\ \mu g.$ P	$\frac{Total\ \Delta\ \mu g.\ P}{mg.\ dry\ weight}$
min.				
0	7.1	—	—	—
10	9.4	2.3	89.7	11.2
26	11.2	4.1	155.8	19.5
40	13.9	6.8	251.6	31.4
50	17.1	10.0	350.0	43.8

Dry weight = 8.0 mg.

in Table I. From determinations made on five samples of uncontracted controls and nine samples of contracted fibers, it may be observed that there is no appreciable loss of water in contraction. The values are to be compared

with the 50 per cent loss of water in contractions as given by Szent-Györgyi (12) for the precipitated type fibers. However, it must be remembered that the surface-spread fibers are crumpled films, with water caught between the folds and without a definite external boundary. The method of determining wet weights is such that roughly the same amounts of water may be caught in the folds of the crumpled film, both in the contracted and uncontracted states. For these reasons, the results must be interpreted with caution.

These fibers possess a definite ATP-ase activity, as shown in the data of Table II. The activity is given as micrograms of inorganic phosphate released per milligram of dry weight. The data are from a typical experiment.

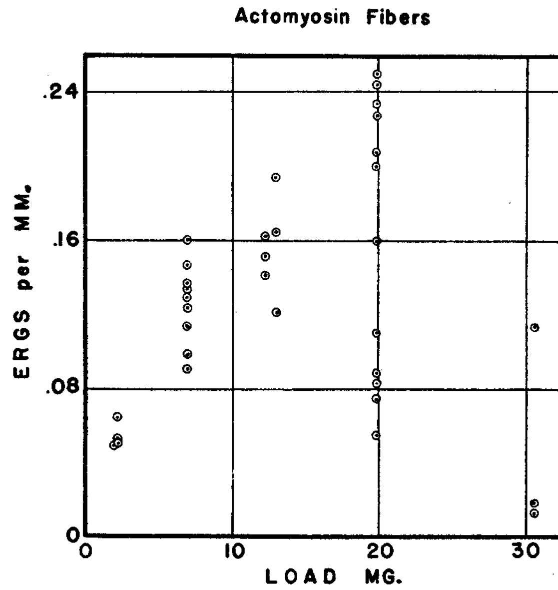


TEXT-FIG. 3. Per cent contraction with load of actomyosin fibers. Each point a 15 minute contraction. Standard conditions as in Text-fig. 2.

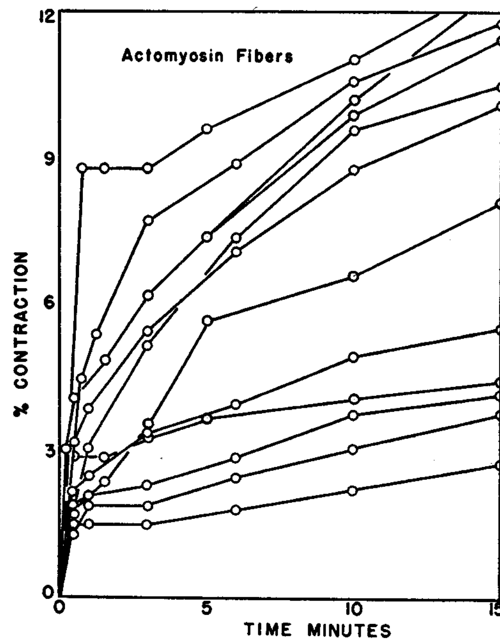
(*b*) *Loaded Contractions.*—Text-fig. 2 depicts graphically the per cent contraction in time of two fibers of approximately equal size loaded with two different weights. The values of all weights given in this and subsequent figures are the values in water, calculated by using a density value of 2.5 for the glass weights. The density of the weights was determined in the usual manner.

The shape of the contraction curves of Text-fig. 2 shows that the fibers begin contracting immediately upon contact with the ATP, and at the most rapid rate. The rate falls off with time, but it may be seen that the contractions are not complete in the 15 minute interval shown. Much higher values of the contractions are obtained in 30 minute or 40 minute contractions.

The effect of the load on the degree of contraction was tested in a series of contractions. Each point in Text-fig. 3 represents a 15 minute contraction with



TEXT-FIG. 4. Work done per millimeter active length of fiber against load. Standard conditions as in Text-fig. 2.



TEXT-FIG. 5. Contraction curves for a load of 20 mg. Standard conditions as for Text-fig. 2.

an individual fiber. The different fibers were formed in as uniform a manner as possible, spreading an excess of actomyosin between barriers placed 75 cm. apart, the fibers being formed with a single sweep of the surface. Nitrogen determinations of the contracting portions of the fibers gave an average protein content of 15.6 μg. N per mm. of fiber, but with a considerable variation which did not always correlate with the performance of the fiber. That is, variation in contraction seems to be due to more than the variable protein content, possibly including differential stretching of portions of the fiber in handling, differences in degree of compression of the film, or other unknown factors. However, even though the points show considerable scatter, it may be seen that the contraction falls off with the load in a regular way, so that with loads of 30 mg. for these fibers, there is very little contraction in 15 minutes. A load of 20 mg. seems to be critical for these fibers, as indicated by the marked scattering of the points.

The same data were recalculated for the relation of the work done to the load (Text-fig. 4). The individual contractions show the same variability as before, but the trend is clearly seen. With increasing load, more work is done per unit length of active fiber, up to the critical load of 20 mg. for this particular group of fibers. The scattering of the points is again evident at the critical load, and may be scrutinized more closely with examination of the shape of the individual contraction curves at this load.

Examination of the contraction curves for the critical load reveals that they seem to be of two types (Text-fig. 5), those which follow the general shape of the normal contraction curve (compare with Text-fig. 2) and those which do not. The latter type seems to result from a brief initial contraction and a subsequent contraction much slower in rate than the normal shaped curves. It would seem that due to the variation of the several fibers, some are capable of sustaining and pulling the critical load in the normal fashion, whereas the other, obviously activated, cannot lift the load.

V

DISCUSSION

The study of such biological properties as contraction in fibers made of actomyosin is an attempt by the investigator to put back together the molecular extracted constituents of torn-down muscle (16). Thus, Weber (14) first announced the formation of solid threads of actomyosin by squirting a solution of the protein through a fine orifice into a precipitating medium, and Szent-Györgyi strikingly demonstrated that these fibers would shorten when treated with ATP (10). However, the work of Perry, Reed, Astbury, and Spark (17) clearly pointed out that this contraction was a colloidal syneresis involving a large loss of water, and Buchtal, Deutsch, Knappeis, and Munch-Petersen (18) showed that the Weber-type fibers, although retaining the capacity to

split ATP, did not contract if the slightest tension was placed upon the fibers. Instead, the fibers under these conditions elongated as a specific effect of the action of ATP. These facts may be interpreted as due to the lack of orientation and intermolecular bonding of the constituent molecules in the precipitated fibers. Szent-Györgyi (15) subsequently demonstrated that lack of orientation was indeed one of the factors by showing that a slight stretching of these fibers would result in contractions not involving the large loss of volume noted by his critics. Neither he nor Weber have claimed structural continuity by a demonstration of the ability of their fibers to lift a load.

The most general qualitative feature of muscle action is the fact that muscle has the ability to transform chemical energy into mechanical work; that is, muscle is able to move a load through a distance. *A priori*, a fundamental requirement of a system capable of such a transformation of energy is that of structural continuity, so that a load may not only be sustained, but pulled through a distance. In terms of actomyosin molecules, therefore, more than orientation is required; a molecule-to-molecule association is also necessary to provide the structure capable of expressing mechanical energy.

Surface-spreading of actomyosin molecules and subsequent compression of the film into fibers provide the necessary intermolecular bonding, as evinced by the ability of these fibers to perform mechanical work (Text-fig. 2).[3] The establishment of such a longitudinally continuous structure is further evidenced in the visible microscopic appearance of the fiber (Fig. 1 *a*) and in the fact that the specific elongation effect of ATP observed by Buchtal *et al.* (18) has never been observed with surface-spread fibers, in the several thousand contractions observed. The fact that such a continuous structure can be established by the simple technique of fiber formation involving surface-spreading of the molecules lends support to the hypothesis that surface-spread protein forms the basis of cellular structures, and justifies the study of the biological properties of intracellular proteins in the surface-spread physical state.

The data on water loss are to be regarded with caution (Table I) since the values of wet weights will vary considerably depending on the method and extent of the blotting of adhering water. Thus, the average value of 90 per cent water content is not to be compared in absolute terms with, say, the water content of intact muscle. On the question of water loss with contraction of these fibers, it is difficult to understand the failure of the fibers to lose water, especially since the diameter of the fiber changes little with contrac-

[3] It may be noted that in March, 1951, when the first note on these findings appeared (5), Weber (19) announced that actomyosin fibers were capable of exerting an isometric tension, but without details of preparation. Since then, Portzehl (20) has published the details of preparation of these Weber-type fibers, which involved precipitation, treatment with glycerine, drying overnight, and stretching.

tion (Fig. 1). It is possible that the contracted fiber (Fig. 1 *b*) is relatively thicker in the plane of the paper than the uncontracted fiber (Fig. 1 *a*) since treatment with ATP causes the fiber to become harder, so that the tendency to become flattened out upon contact with the slide is considerably less than is the case with the uncontracted fiber. Again, the explanation may lie in the fact that the method of wet weight determination does not distinguish between the water in the film proper and the adhering water or the water trapped between the folds of the film, and such water, in a system with no definite external boundaries with regard to the surrounding aqueous medium, may remain fairly constant in the uncontracted and contracted states. Whatever the explanation, the fact remains that no sizeable loss of water occurs, and this at least lends support to the idea that, rather than colloidal syneresis, the *modus operandi* of the contractions is a configurational change in structure.

The surface-spread actomyosin has a readily measurable ATP-ase activity, as shown in Table II. If we assume that the energy of the terminal bond of the ATP is being utilized to perform the work done by the fiber, a simple calculation will show that with the activity of Table II, the fiber splits enough ATP in a fraction of a second to perform the work of a 10 minute contraction. There is, of course, the possibility that contraction does not result from the splitting of the ATP but from another type of reaction between the actomyosin and ATP (21).

This leads us to a consideration of the rate of contraction of the fiber, which is of the order of 10^4 times slower than the contraction of striated muscle. Two possible explanations of the mechanisms may be advanced to account for this slowness:

(*a*) Since, for the fibrous system, the activating agent ATP is added from outside the fiber, the rate of diffusion of the ATP may be the limiting factor in determining the rate of contraction. Hill (22) has shown that the time required for diffusion in this type of system is proportional to the square of the radius of the fiber, and for one of a diameter equal to 0.5 mm., time of the order of 10 to 15 minutes would not be unreasonable. This would mean that a continuing reaction during the course of the contraction is required for the full contraction, and if this reaction should involve the terminal bond energy, it would mean that more than 99 per cent of this energy would be wasted as heat.

(*b*) The second mechanism assumed that the fiber reacts rapidly with the ATP upon initial contact with the solution; that is, most of the actomyosin molecules in the fiber are exposed to immediate contact with the ATP, so that diffusion is not a limiting factor. From this initial reaction, the surface-spread actomyosin undergoes a configurational change to bring about contraction. If this view is accepted, then some unknown condition, such as the

imperfection of the structural organization of the fiber must be invoked to account for the slowness of the contraction.

At present, a decision between these mechanisms, or indeed any other mechanism, is not possible, although some suggestive data are available. Thus, the curves of Text-fig. 5 indicate that when the load of 20 mg. is too heavy for the particular fiber, the fiber essentially stops contracting after the first few moments of activation. Other experiments (soon to be published) show that the fiber under these circumstances is not inactivated, and if the load be removed, contraction takes place normally. If a continuous reaction with ATP limited by the diffusion rate of ATP were the mechanism of contraction, one would expect either a smooth regular curve of limited contraction, or a curve of the type obtained, but accompanied by an inactivation of the contractile elements. Therefore, these data would support the second of the two mechanisms suggested above.

VI

SUMMARY

1. Surface-spread actomyosin, compressed into fibers, shows biological properties of contractility and enzymic activity.

2. In unloaded contractions, wet and dry weight determinations show no appreciable water loss in contraction. The fibers also evince a strong ATP-ase activity.

3. A structural continuity in the fibers by intermolecular linkages of the component actomyosin molecules is established during the formation of the fibers. Evidence includes their visible longitudinal structural organization, the lack of elongation effect of ATP when under tension, and their ability to lift appreciable loads, so that, like muscle, they can transform chemical energy into mechanical work.

4. Up to a limiting critical weight, the fibers perform more work with increasing imposed weight load.

5. Theoretical aspects are discussed, including the possibility that surface-spread protein is involved in the formation of cell structures. Possible explanations for the relative slowness of the fiber contractions are offered.

It is a pleasure to acknowledge my indebtedness to Miss Raja Rosenblueth and Mr. George Edison, whose cooperation and enthusiasm not only made this investigation possible but also enjoyable, to Dr. Alexander Sandow for stimulating discussion, and Mrs. Virginia Thorndike for checking of the manuscript.

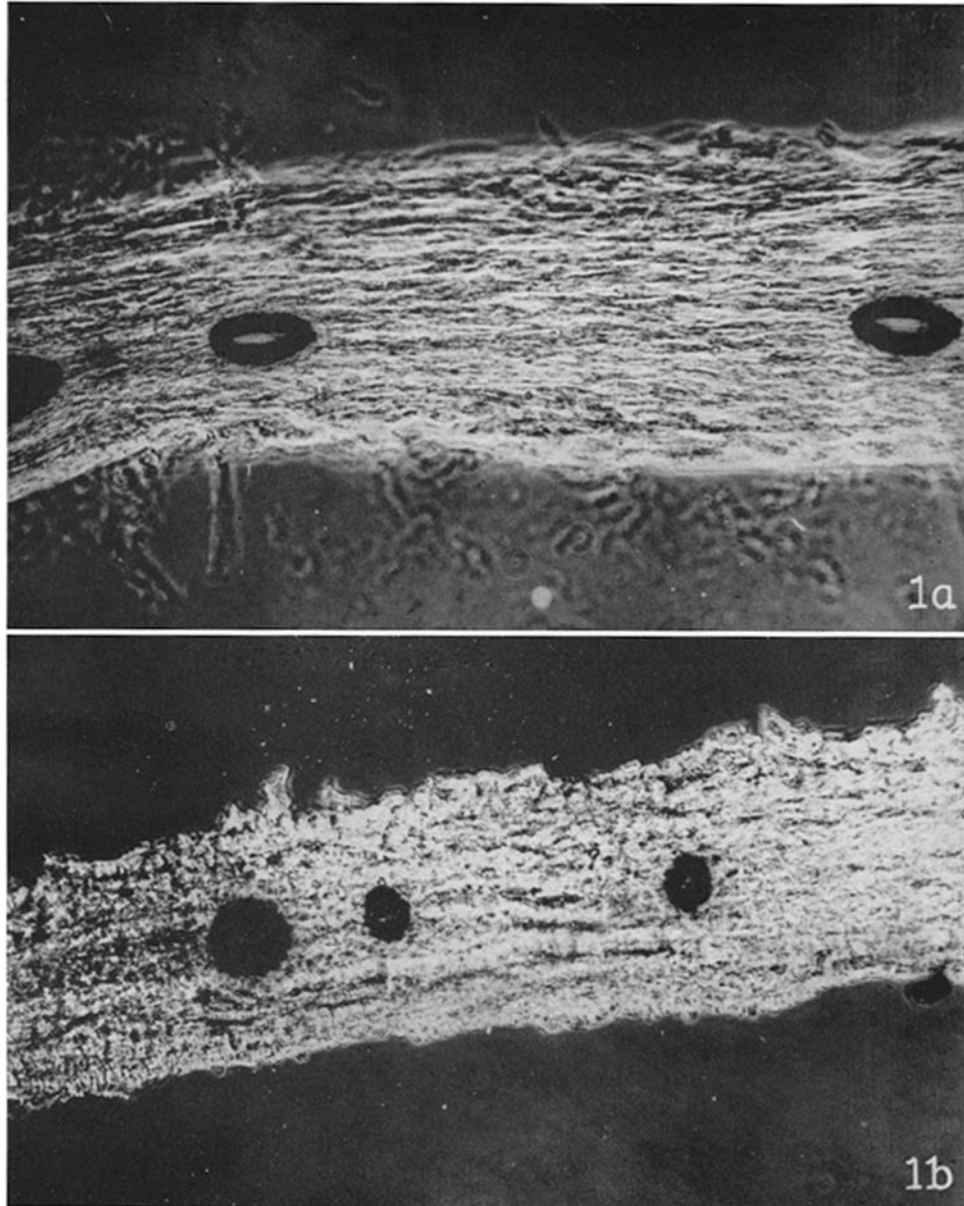
BIBLIOGRAPHY

1. Mazia, D., Hayashi, T., and Yudowitch, K., *Cold Spring Harbor Symp. Quant. Biol.*, 1947, **12**, 122.
2. Hayashi, T., and Edison, G. R., *J. Colloid Sc.*, 1950, **5**, 437.

3. Hayashi, T., *Science*, 1951, **114**, 684.
4. Kaplan, J. G., *Fed. Proc.*, 1950, **9**, 69.
5. Hayashi, T., *Fed. Proc.*, 1951, **10**, 61.
6. Hall, C. E., Jakus, M., and Schmitt, F. O., *Biol. Bull.*, 1946, **90**, 32.
7. Schmitt, F. O., Bear, R. S., Hall, C. E., and Jakus, M. A., *Ann. New York Acad. Sc.*, 1947, **47**, 799.
8. Engelhardt, W. A., and Ljubimowa, M. N., *Nature*, 1939, **144**, 668.
9. Buchtal, F., Deutsch, A., Knappeis, G. G., and Munch-Petersen, A., *Acta physiol. scand.*, 1949, **16**, 326.
10. Szent-Györgyi, A., *Studies Inst. Med. Chem., Univ. Szeged*, 1941, **1**, 17.
11. Munch-Petersen, A., *Nature*, 1948, **162**, 537.
12. Szent-Györgyi, A., Chemistry of Muscular Contraction, New York, Academic Press, 1947.
13. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Related Methods for the Study of Tissue Metabolism, Minneapolis, Burgess Publishing Co., 1945.
14. Weber, H. H., *Ergebn. Physiol*, 1934, **36**, 109.
15. Szent-Györgyi, A., *Biochim. et Biophysic. Acta*, 1950, **4**, 38.
16. Szent-Györgyi, A., Chemistry of Muscular Contraction, New York, Academic Press, 2nd edition, 1950.
17. Perry, S. V., Reed, R., Astbury, W. T., and Spark, L. C., *Biochim. et Biophysic. Acta*, 1948, **2**, 674.
18. Buchtal, F., Deutsch, A., Knappeis, G. G., and Munch-Petersen, A., *Acta physiol. scand.*, 1947, **13**, 167.
19. Weber, H. H., *Nature*, 1951, **167**, 381.
20. Portzehl, H., *Z. Naturforschung*, 1951, **6b**, 356.
21. Mommaerts, W. F. H. M., Muscular Contraction, New York, Interscience Press, 1950.
22. Hill, A. V., *Proc. Roy. Soc. London, Series B*, 1928, **104**, 39.

EXPLANATION OF PLATE 2

FIGS. 1 *a* and 1 *b*. Photomicrographs of actomyosin fibers. (*a*) Uncontracted. (*b*) Contracted. Low power (× 93), phase contrast.



(Hayashi: Contraction of surface-spread actomyosin)