THE EFFECT OF POLYPHOSPHATES AND MAGNESIUM ON THE MECHANICAL PROPERTIES OF EXTRACTED MUSCLE FIBERS

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Living muscle fibers at rest have high extensibility, similar to that of rubber. However, no final conclusions can be drawn from this observation because supporting structures may determine the mechanical properties of muscle when examined with static methods. In the glycerol-extracted muscle fibers introduced by Szent-Györgyi (1) mechanical properties can be studied under a great variety of conditions, because the composition of the medium surrounding the myofibrils can be readily controlled owing to the breakdown of the cell surface. This makes it possible to modify the physical properties of these fibers within wide limits. Because contractility remains intact, such studies may throw light on some of the essential characteristics of muscle.

The observations on extracted muscle fibers reported here indicate that, all appearances to the contrary, myofibrils, even at rest, have short range elasticity, like muscle in rigor, and that extension is largely a process of plastic deformation. These conclusions are based on an analysis of the softening action of polyphosphates and on the observation that under certain conditions contraction may be induced by ATP[1] without an appreciable change in extensibility and elasticity. Additional evidence confirmed that PP and ATP cause softening and partial relaxation only in the presence of Mg and that these effects do not require a relaxation factor (2, 3).

Methods.—Psoas muscle from young rabbits, preserved at their length *in situ* in 50 per cent glycerol at −15° was used. Fine strands of fibers were mounted in the form of a loop (about 2.2 cm. long) in a chamber as described previously (4). The chamber was immersed in a bath which usually had a temperature of about 8°.

To study elastic and viscous properties separately, muscle fibers were suddenly extended by withdrawing support from a load and then unloading again suddenly by the reverse process. The changes in length were recorded by an isotonic lever. In comparison with previous technique (5), the procedure was improved firstly by providing conditions which make possible more complete penetration of ATP into the fibers, ensuring that mechanical conditions inside the fibers were uniform. This

[1] The following abbreviations will be used, ATP for adenosinetriphosphate, PC for phosphocreatine, PP for inorganic pyrophosphate, EDTA for ethylenediaminetetraacetate.

was achieved by using thinner preparations, subdividing them into several strands, and by low temperatures. Secondly, the connection to the lever was made more rigid by using a light chain in place of a thread. To test rigidity the chain was directly connected to the chamber. Loading then produced extension amounting to about 10 per cent of that obtained with muscle fibers. The measurements were corrected for this effect. The natural period of the lever, when connected to a preparation was 14 vibrations per second, adequate for the recording speeds used, as shown by tests using fibers in rigor (Figs. 3 and 8).

Because elastic extension of muscle fibers is very small, slack is a serious source of error. To minimize it, a small load, producing a tension of 0.4 gm. (about 120 gm. per cm.^{2} cross-section), was attached to the lever throughout the experiment. Nevertheless, elastic extension by an increase in load was large at the beginning of an experiment and diminished when the fibers were loaded and unloaded several times. This effect, which probably was caused by slack in some of the fibers in a preparation, was abolished if previously a moderate load was applied for several minutes or an isometric contraction was produced by ATP. The elastic effects then were remarkably constant and were reproducible within ± 10 per cent. Shortening on unloading could be measured more accurately than extension by a load. The former, therefore, was used for determining elastic effects.

After the sudden elastic extension and a brief and small phase of delayed elastic extension a load produced a further slow lengthening which was not reversed after unloading and which will, therefore, be described as plastic. The rate of this extension, given by the slope of the extension time curve, will be used as a measure of the resistance to plastic extension.

In a second type of experiment the change in tension produced by stretching and releasing was measured. The muscle chamber was mounted on a microscope stand. The preparations, which were under tension initially, were rapidly stretched or allowed to shorten a predetermined length by turning the fine adjustment screw, which was provided with stops. The preparations were connected vertically to an isometric lever by a chain. Tension was measured by an optical system with an optical lever of about 2 meters and read on a scale. Although the system was made as rigid as seemed possible, it had a "give" which reduced the actual change in length below that read from the screw. A correction, amounting to 25 to 35 per cent, was necessary to eliminate this error; it was determined by connecting the chain directly to the muscle chamber. The natural period of the lever was 24 vibrations per second. In some experiments tension was recorded on a smoked drum.

The change in tension after a rapid stretch was measured about 1 second after the change in length. In the absence of polyphosphates this value represented essentially elastic tension because elastic after effects were rather small. In the presence of high concentrations of ATP, however, tension changed rapidly in the first second owing to plastic deformation, later more slowly. The values obtained, therefore, were largely determined by resistance to plastic stretch. The results of such experiments will be expressed in terms of rigidity, which is defined like elastic modulus except that tension was measured 1 second after stretch. In successive stretches the results were reproducible within ± 5 to 12 per cent depending on the degree of extensibility. The values reported here are averages of 2 to 4 measurements. Cross-sectional area

was computed from the weight of the preparations, determined at the end of the experiment on a torsion balance after blotting with filter paper. Specific gravity was assumed to be 1.05.

The effect of incomplete penetration of ATP (8) resulting from its rapid breakdown should be discussed briefly. According to Hasselbach's results (8) 10 mM ATP penetrates about 20 μ into psoas fibers at 8°. This is adequate because the diameter of the fibers is on the average about 30 μ. At the lower concentrations, however, the softening effect of ATP probably would have been stronger if penetration had been complete. Since under the conditions of these experiments absence of Mg diminishes enzymatic activity, it will improve penetration. This should tend to diminish the effects of Mg described. In the presence of a high concentration of PC, ATP may be expected to penetrate well because, owing to rapid rephosphorylation, only small amounts of ATP are utilized. The high tension produced testifies that the whole fiber participates in the contractions.

All experimental solutions contained 0.14 M KCl and 0.02 M phosphate buffer of pH 7 and, unless specifically stated, 2 mM $MgCl_2$. All other solutions were likewise adjusted to pH 7. ATP was obtained as the Na salt from Pabst Laboratories, Milwaukee, PC from Sigma Chemical Co., St. Louis.

RESULTS

A. Mechanical Properties during Rigor.—Muscle fibers preserved in glycerol and washed in KCl solution are in a state of rigor. Light or moderate loads produced a sudden, reversible change in length, followed by a small elastic after-effect. With heavy loads the purely elastic extension was followed by a very slow extension which continued for long periods. Elastic extension was somewhat larger in proportion to tension for small than for large loads (Fig. 1), but this small deviation from Hooke's law probably was, at least in part, due to slack in part of the fibers or a mechanical defect in the recording system. The elastic modulus computed from the sudden change in length at the natural length of the fibers was in 18 preparations on the average 6.2×10^7 (range 4.2 to 7.2×10^7) dynes/cm.^{2}, about 40 times larger than that of rubber, if compared on a water-free basis.

Contrary to expectation, elastic extension was not significantly influenced by large changes in length. The fibers which were immersed in 8 mM ATP solutions, sometimes containing PC (at 6° to 8°), were washed in KCl solution after they had shortened to various lengths and finally slowly reextended by a load, while immersed in 3 mM PP. The results were the same during shortening and lengthening. It was found that, within the limits of the accuracy of the method, a given load produced the same elastic extension regardless of length (Fig. 2). A possible explanation for this result will be given below.

A marked increase in elastic extensibility can be produced as follows. Fibers, from which about half of the bound Mg or Ca has been removed by washing in, respectively, 2 mM PP or 5 mM EDTA, swell reversibly in distilled water more than 100 per cent and shorten about 5 per cent of their length (6). Further-

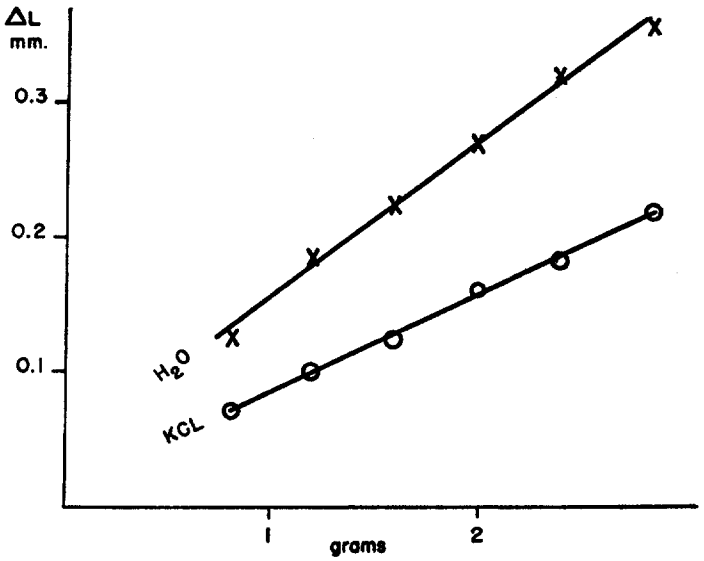

FIG. 1. Elastic extension as a function of load, determined first while fibers were in 0.16 M KCl (circles), then after previous treatment with PP to remove bound Mg and swelling in water (crosses). 8°.

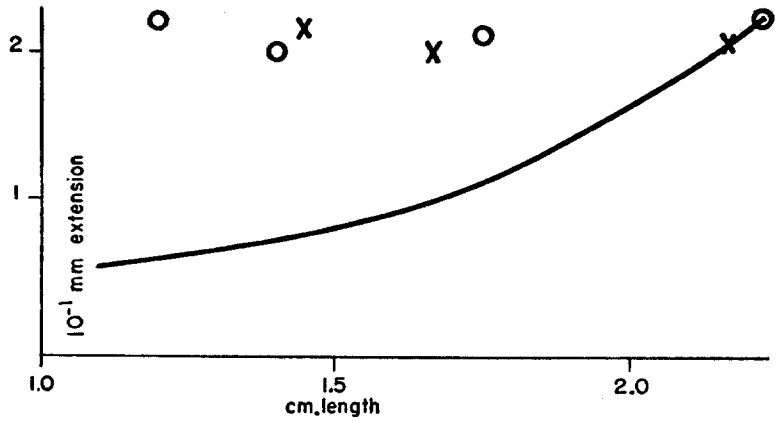

FIG. 2. Elastic extension at different lengths. Fibers were first allowed to shorten to different lengths in a solution containing 10^{-4} M ATP and 2.10^{-2} M PC per liter (circles). Then they were slowly stretched by a load while immersed in 3 mM PP. Elastic extension by a load of 1.67 gm. was determined while fibers were in 0.16 M KCl. Line represents values expected if elastic modulus had remained constant. 10°.

more, light scattering is strongly decreased, indicating that water is taken up inside the myofibrils. In such fibers elastic extension was increased 60 to 80 per cent (Fig. 1). To explain this phenomenon it may be assumed that Ca and Mg form cross-links and that their partial removal permits Donnan swelling.

Because of increased lateral distance the chain molecules then are able to curl and thereby shorten slightly.

B. Effect of PP on Elasticity and Plastic Extension.—Bozler (5) found that in the presence of PP a load produced a small elastic extension, followed by a gradual lengthening. The last of these effects cannot be described as damped elasticity, because the length of the fibers was not restored after unloading except by active contraction. Conversely, after shortening in ATP solutions, these fibers did not return to their original length unless an external force was applied. Therefore, the fibers may be described as being plastic.

Elastic and plastic extension were studied quantitatively in fibers which had been allowed to shorten in ATP solutions to about two-thirds of their length *in situ*. In such fibers moderate loads gave an extension time curve which was linear over a narrow range of lengths. Therefore, the loads were applied only long enough to permit measurement of the slope of the extension time curve,

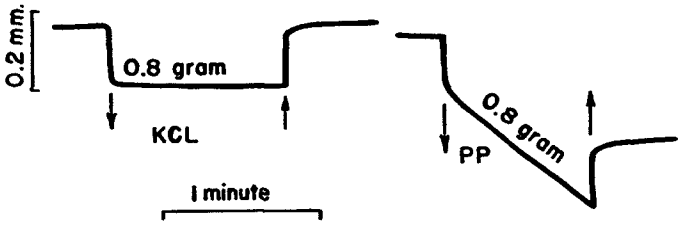

FIG. 3. Elastic and plastic extension produced by a sudden increase in load (0.8 gm.). Loading and unloading indicated by arrows. Left: fibers in KCl solutions; right: fibers in 2.0 mM PP containing $MgCl_2$ (2 mM per liter). The curves are enlarged copies of kymograph records. 8°.

which measures resistance to plastic extension, but so that the fibers were not stretched more than about 2 mm. during a whole experiment. It was found that PP increased the slope of the extension time curve many times, but had no effect on the purely elastic extension (Figs. 3 and 4). Thus softening by PP was entirely due to a lowering of resistance to plastic extension. Because of other similarities, ATP could be expected to have essentially the same effect on mechanical properties as PP. This view was confirmed by experiments which will be described below.

C. Effects of ATP.—In studies on the effect of ATP, rigidity, as determined by the method mentioned above, was convenient as an index of changes in mechanical properties. Near the physiological range of concentrations, ATP strongly diminished rigidity (Fig. 5). It has been shown previously (7) that even much lower concentrations of ATP produce a strong contraction if PC is present. As shown in the experiment illustrated in Fig. 5, rigidity remained nearly unchanged under these conditions, although as much or more tension was produced than by high concentrations of ATP. It may be concluded that the softening effect of ATP is entirely independent of contraction itself.

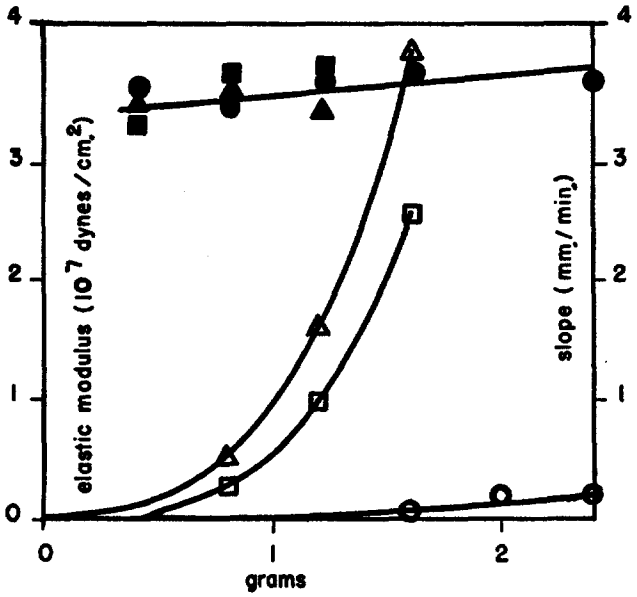

FIG. 4. Effect of PP on elastic modulus and resistance to plastic extension. Abscissa, load; ordinate, slope of extension time curve. The same fibers were successively immersed in 0.16 M KCl (circles), 1.0 mM PP (squares), 2.0 mM PP (triangles). Full signs, modulus; empty signs, slope of extension time curve. 8°.

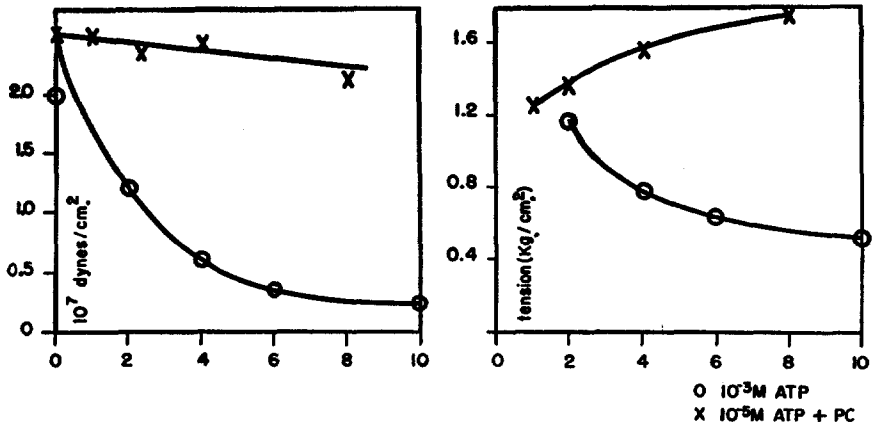

FIG. 5. Effect of concentration of ATP on rigidity (left) and maximal tension (right). Crosses: fibers in low concentrations of ATP, containing 20 mM PC per liter. Circles: high concentrations of ATP without PC. 8°.

These results were confirmed by experiments at low temperatures. At 0° high concentrations of ATP made the fibers even softer than at higher temperatures, whereas low concentrations with added PC, which produced about twice

as much tension, had almost no effect on rigidity (Fig. 6). Therefore, during contraction the mechanical properties may remain almost exactly like those during rigor.

D. Role of Mg.—Contraction without appreciable softening can be produced also by another procedure suggested by previous findings on the role of Mg. Bozler (2, 6) has shown that tightly bound Mg masks some of the effects of this metal. Removal of part of this bound Mg by repeated washing in solutions of PP abolished the softening action of PP and diminished that of ATP at 0°. Immersion in solutions containing $MgCl_2$ restored these effects, demonstrating the requirement for Mg.

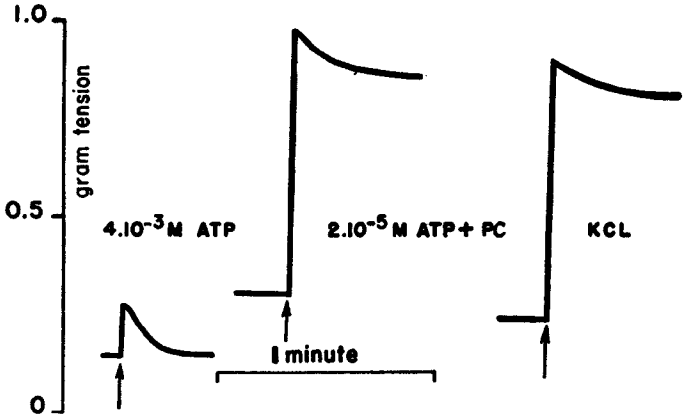

FIG. 6. Effect of ATP on rigidity at 0°. Change in tension produced by stretch (arrow) was recorded on smoked drum. Left: fibers were in 4.10^{-3} M ATP and were stretched 0.1 mm. Middle: fibers in 2.10^{-5} M ATP, containing 20 mM PC per liter, were stretched 0.04 mm. In the last of these solutions the fibers produced actively more than twice as much tension as in the former, but they remained nearly as inextensible as in KCl solution. Right: fibers in 0.16 M KCl stretched 0.1 mm.

That Mg in the presence of ATP produces softening, under certain conditions also partial relaxation, is shown in the experiments illustrated in Figs. 7 and 8. To remove bound Mg, muscle fibers were washed alternately three times in 2 mM PP and 0.16 M KCl, both without $MgCl_2$. ATP solutions without $MgCl_2$ then produced strong contractions, but changed rigidity only slightly, whereas later ATP solutions containing Mg (2 mM $MgCl_2$ per liter) diminished rigidity several fold. Addition of $MgCl_2$ increased maximal tension at low concentrations, but lowered it at very high concentrations of ATP (Fig. 7).

In separate experiments, PP-treated preparations were first brought into Mg free 10 mM ATP (at 10°). After tension had reached its maximum, the addition of $MgCl_2$ (2 mM per liter) caused tension to drop rapidly by 15 to 32 per cent (4 experiments). At lower ATP concentrations $MgCl_2$ produced a rise in tension. These experiments clearly demonstrate that the softening and relaxing

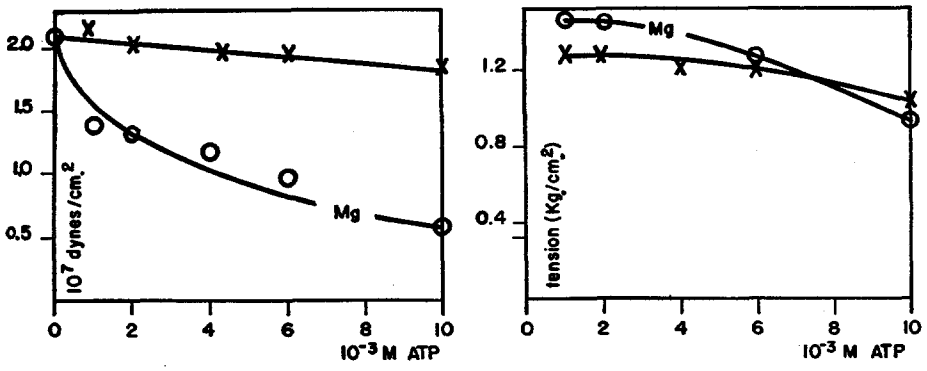

FIG. 7. Effect of concentration of ATP and Mg on rigidity (left) and maximal tension (right). Crosses: fibers, which had been previously treated with PP to remove bound Mg, in Mg-free ATP solutions. Circles: same fibers later in ATP solutions containing $MgCl_2$ (2 mM per liter). 8°.

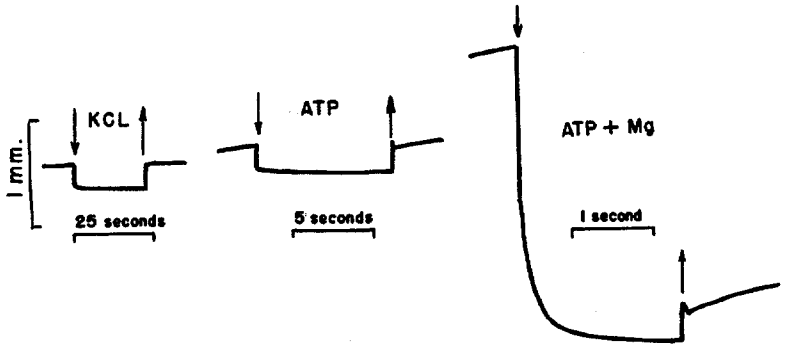

FIG. 8. Elastic and plastic extension as influenced by ATP and Mg. Loading (1.2 gm.) indicated by arrows. The fibers (21 mm. long) were first treated with PP to remove part of bound Mg and then immersed in Mg-free KCl solutions (left). Muscle fibers were then allowed to shorten to 19 mm. in Mg-free 4 mM ATP. The load produced slow plastic extension (middle). After unloading, $MgCl_2$ (2 mM per liter) was added (right), producing a more rapid contraction. After shortening to 17 mm., the load produced very rapid extension. Note differences in time scale. The elastic shortening on unloading remained the same under all conditions. The curves are enlarged copies of kymograph records. 7°.

action of ATP depends on the presence of Mg. It is significant also that these effects of Mg were obtained in old fibers and, therefore, did not depend on the presence of a relaxation factor.

As pointed out above, penetration of ATP into the fibers probably was incomplete at low concentrations of ATP, but this does not invalidate the results. On the contrary, because absence of Mg, by lowering the breakdown of ATP,

increases the depth of penetration and Mg increased the softening action of ATP, incomplete penetration of ATP would tend to diminish the effects described.

In the experiment illustrated in Fig. 8 elastic and plastic properties were studied separately. Muscle fibers, previously treated with PP to remove part of the bound Mg, were allowed to shorten in a Mg-free ATP solution. Plastic extension by a load was very slow, but after adding $MgCl_2$ it became so fast that elastic and plastic extension merged and half the increase in length was completed in less than 0.1 second. However, the purely elastic changes in length on unloading remained the same as in rigor under all these conditions.

DISCUSSION

A.—The mechanical properties of living skeletal muscle have been studied extensively. In single relaxed muscle fibers no clear separation of elastic and viscous or plastic effects was observed by Buchthal and Kaiser (9) in loading experiments similar to those reported here, probably because plastic extension was too rapid. With a vibration method these authors (p. 84 of reference 9) found at 20° a dynamic modulus of 2.5×10^6 dynes per cm.^{2} at rest, twice as much during contraction, values which are, respectively, 24 and 12 times smaller than those reported here. As pointed out by Hill (13), plastic extension may produce a considerable error in this method. Boukaert *et al.* (10) observed that in contracted muscle an increase in load produced a considerable, sudden extension, which diminished strongly with increasing initial load (11), followed by a more gradual adjustment in length. However, as pointed out by Hill (12, 13), earlier experiments generally were vitiated by an appreciable "give" in the recording instruments. On the basis of thermoelastic studies he concluded that contracted muscle has only short range elasticity.

Washed muscle fibers have the advantage over living muscle that the mechanical properties of the myofibrils can be varied reversibly within wide limits. On one extreme, in rigor and in some states of contraction, the fibers are almost perfect elastic bodies. On the other extreme, in the presence of physiological concentrations of ATP and of Mg, particularly at low temperatures, extension by a load is mainly plastic, but so rapid that elastic and plastic components merge. If the latter condition, which mechanically resembles relaxed living muscle, were studied alone, the fiber might appear to have rubber-like, but damped, elasticity. However, the existence of a distinct elastic component with a high stretch modulus is demonstrated by the fact that in the same fibers all transitions between rigor and relaxation can be produced, that PP softens without change in elasticity, and particularly by the observation that on unloading the elastic shortening is the same under all these conditions. These results, furthermore, indicate that extension of muscle is mainly due to plastic deformation.

That elastic extension by a load is independent of length is surprising. A possible explanation for this observation is the assumption that elastic extension is due to a structure in series with the contractile elements. In this case an even higher elastic modulus would have to be assumed for the contractile elements than indicated by the present experiments. Hill (12) has previously postulated a series elastic component in contracted muscle and considered it to consist, at least in part, of tendons.

B.—The strength of contraction diminishes beyond a certain optimal concentration of ATP, which varies inversely with temperature (14). This partial relaxation is associated with a decrease in ATP breakdown only at concentrations above about 8 mM per liter and, therefore, is only partly due to diminished enzymatic activity. It is probably caused by the softening action of ATP, in agreement with previous studies on smooth muscle and extracted muscle fibers, which indicate that the energy expenditure for maintaining tension is determined by resistance to plastic extension (4, 15). Thus the drop in tension produced by superoptimal concentrations of ATP basically is very different from normal relaxation.

Bozler (7, 16), Bendall (18), and others have described a condition resembling normal relaxation more closely. In this state, which requires the presence of Mg, tension is lowered further, enzymatic activity is diminished many times, and $CaCl_2$ in low concentrations causes strong contraction. Mg is necessary for relaxation partly because it is essential for the softening action of ATP. However, this is not a complete explanation, because Mg not only softens, but, under appropriate conditions, also inhibits the breakdown of ATP (7), two effects which seem to be independent of each other. Relaxation, furthermore, involves an additional mechanism, as shown by the requirement for one of several relaxation factors (18–20). Studies on the effect of EDTA by Bozler (3) and Watanabe (21) have suggested that this mechanism is the inactivation of bound Ca. The action of Ca is not understood. As shown previously (2) Ca does not block the softening action of polyphosphates. The observation (17) that very low concentrations of Ca produce maximal activation and that an increase in the concentration of Mg has no inhibiting effect, furthermore, shows that Ca and Mg are not competitive and, therefore, apparently act on separate mechanisms.

C.—As pointed out by Pryor (23), contraction must be associated with increased elastic extensibility if it is caused by an increase in configurational entropy, as assumed by many recent investigators (22–24). In the contraction cycles produced in collagen fibers by Pryor and in muscle fibers by Laki and Bowen (25) the fibers indeed become rubber-like. Also, the cycle described above in which contraction and relaxation were obtained by changing ionic strength shows this effect. These types of contraction, therefore, are funda-

mentally different from the contraction induced by ATP, in which elastic extensibility remains unchanged.

SUMMARY

Loading of extracted muscle fibers causes a small, sudden lengthening, followed by a slower, plastic extension, which is reversed only by active contraction. Polyphosphates in the presence of Mg strongly accelerate plastic extension, but elastic changes in length remain the same as during rigor. The modulus of elasticity on the average is about 6.2×10^7 dynes per cm.^{2} This value is about 40 times larger than that of rubber, if compared on a water-free basis. Extension of muscle, therefore, is almost entirely due to plastic deformation.

Mg is essential for the softening action of adenosinetriphosphate (ATP) and can produce partial relaxation in the absence of a relaxation factor. After partial removal of bound Mg, ATP causes strong contraction, but only slight softening. The same condition is produced by very low concentrations of ATP in the presence of phosphocreatine. These observations show that during contraction passive mechanical properties may remain essentially like those during rigor. The constancy of elastic extensibility distinguishes contraction produced by ATP from contraction induced by non-specific agents in various fibrous structures and caused by an increase in configurational entropy.

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