

## THE EFFECT OF ELECTROLYTES ON THE CONTRACTILE ELEMENTS OF MUSCLE

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If it is assumed that electrostatic attraction (5) or repulsion (4, 7, 8, 11) between charged groups of proteins is responsible for muscular contraction or relaxation, it may be expected that electrolytes have a strong effect on the properties of contractile proteins and that possibly contractile phenomena can be produced by their action (2). This question cannot be studied in intact muscle fibers, but glycerol-extracted muscle fibers as described by Szent-Györgyi (10, 11) are very suitable for this purpose. In experiments on such preparations it was found that a contraction is indeed produced by washing out electrolytes. This procedure increases the total charge of the protein and probably causes shortening because of the electrostatic attraction between adjacent charged groups. It is difficult to reconcile this finding with the assumption, which is made in most recent discussions on the mechanism of muscular contraction, that the electric charge of polar groups tends to keep protein molecules distended, producing relaxation.

However, the contraction described does not explain normal contraction because it permits only a small degree of shortening. These results illustrate some of the difficulties of the electrostatic theory in its present form, but they do not exclude the possibility that attraction between adjacent charged groups is part of the normal mechanism of contraction. Possibly ATP permits the successive interaction between more and more distant groups.

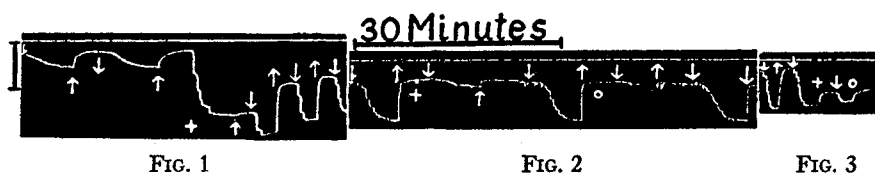
### *Procedure*

The experiments generally were carried out with muscles which have been stored in 50 per cent glycerol for from 1 day to several weeks as described by Szent-Györgyi (11, see also 1). Several types of muscles were studied, smooth muscle (rings from the frog's stomach, longitudinal muscle of the small intestine of the dog and cat), cardiac muscle (small strips from the auricle and ventricle of cats and rabbits), and skeletal muscle (m. psoas of the rabbit). The latter preparation, introduced by Szent-Györgyi, was by far the most convenient of the preparations and was used in most experiments, but the other muscles gave the same results. Previous treatment with glycerol is not essential. Preparations which had been washed in 0.001 M/liter  $MgCl_2$  for a few hours at 5° gave exactly the same responses to ATP and electrolytes.

For experiments a thin strand of the psoas, with a cross-section of about 0.1 to 0.2 mm., was isolated and usually, to facilitate diffusion, separated into 2 or 3 still finer strands. A loop, about 14 mm. long, was formed, the open end of which was tied to a fine thread connected to a muscle lever. The lower end of the loop was held by a wire inside a small chamber. Changes in tension or length were recorded on a smoked drum. The temperature was 21–26°. The fibers were immersed in an isotonic KCl solution at the beginning of an experiment. ATP was prepared as the K salt from the commercial Ba salt. It was diluted with isotonic KCl solution.

#### RESULTS

If a muscle preparation which had been in 0.16 M/liter KCl is brought into distilled water, tension rises. Addition of KCl causes rapid relaxation (Fig. 1). This cycle of contraction and relaxation can be repeated as often as desired for



FIGS. 1, 2, and 3. Records of isometric contractions of psoas preparations. Arrow down, immersion in water; arrow up, 0.16 M/liter KCl. Ordinate, 1 gm.

FIG. 1. Effect of washing out electrolytes before and after applying 0.25 per cent ATP (marked by +).

FIG. 2. At + a KCl solution containing 0.01 molar  $MgCl_2$ , at O a KCl solution containing 0.01 molar  $CaCl_2$  were applied, after which the contraction caused by water was greatly reduced. KCl solution restored the previous condition.

FIG. 3. At + a 0.003 molar KCl solution was applied, at O, 0.00075 molar  $MgCl_2$ .

several hours. The magnitude of the effect and the speed of the rise in tension can be influenced by the following conditions.

1. After a preparation has been immersed in a 0.2 per cent solution of adenosinetriphosphate (ATP), washing out the electrolytes causes a stronger contraction and maximal tension is reached more rapidly than before (Fig. 1). This enhancing effect of ATP, which persists for several hours, is not the result of a higher initial tension. If time is allowed for the tension to drop or if the tension is released by slight shortening, the effect persists.

If, after the peak of an ATP contraction is reached, the muscle is washed in water, there is an additional rise in tension, but the tension is not well maintained (Fig. 1, 6).

The tension produced in water after previous treatment with ATP may amount to more than one-third the maximal tension produced by ATP (about 500 gm. per  $cm.^2$  cross-section). In preparations of the frog's stomach it is nearly half as great as the tension during a spontaneous contraction of the fresh muscle.

2. Effect of valency of ions. The effect depends very strikingly on whether the solution used contains monovalent or divalent cations. No difference was found between the effect of NaCl and KCl. If, on the contrary, a preparation was brought into a solution of  $MgCl_2$  or  $CaCl_2$  (0.12 M/liter), subsequent washing with water did not cause a rise in tension, but this response was fully restored after brief immersion in a solution of NaCl or KCl. After bathing in a solution containing 0.15 M/liter KCl and 0.01 M/liter  $MgCl_2$  or  $CaCl_2$ , washing in water caused only a small rise in tension, more if  $MgCl_2$  than if  $CaCl_2$  was

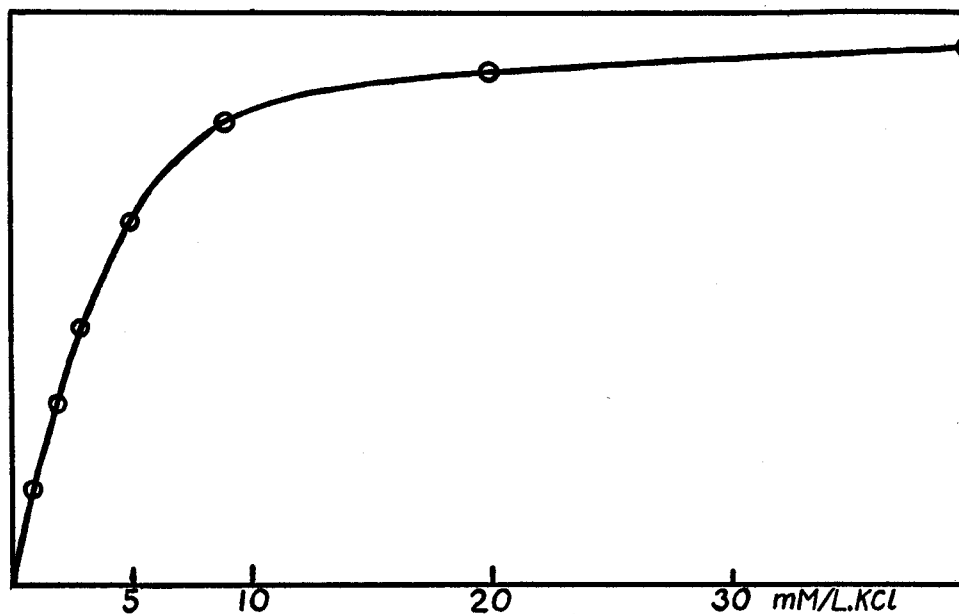


FIG. 4. Drop in tension of a preparation in water on application of different concentrations of KCl.

used (Fig. 2). These observations suggest that divalent cations cannot be removed by water but that they readily exchange with monovalent ions. Replacing monovalent by divalent ions or *vice versa* has no effect on tension.

Tension rises only when the concentration of KCl is less than 0.04 M/liter. The relation between concentration of ions and tension was studied quantitatively by adding a KCl solution to a preparation in which a contraction had developed in distilled water (Fig. 4).

Adding  $MgCl_2$  to distilled water lowers the tension of a muscle more than a corresponding amount of KCl, but the effects of these salts could not be compared accurately because  $MgCl_2$  produces at first a rapid drop in tension like KCl, but later tension slowly declines still further (Fig. 3). At the same Na ion concentration NaCl and  $Na_2SO_4$  had nearly the same effect.

Contraction can be explained as being due to an increase in total charge (see below). If net charge were also important, changes in pH should cause contraction or relaxation. It was found, however, that the muscles remained relaxed if pH was varied between 5 and 8 by phosphate buffers. It seemed possible that in this type of experiment the effects of pH were overshadowed by the presence of large amounts of ions. Therefore, in another type of experiment, buffers were avoided and small amounts of weak acids (acetic acid, lactic acid) or bases (ammonia, nicotine) were added to distilled water. The tension which had developed in water dropped, whether pH was increased or decreased, in all effective concentrations. This occurred when pH was greater than 8.5 or less than 5.5. At the same time the preparations were irreversibly damaged as shown by the fact that the effects of removing electrolyte were permanently reduced or abolished. Also the contraction induced by ATP was diminished, and to about the same degree. The drop in tension caused by dilute acids and bases probably is partly due to their injurious effect, partly to the presence of ions.

The rise in tension in water is associated with two other physical changes. (1) The preparations, which are opaque in saline, become very transparent and appear glassy in water. (2) They swell markedly. These effects, like the rise in tension are rapidly reversed by the presence of electrolytes. Although the water content may more than double in water, all, or most of it, is given up within a few seconds in 0.16 molar KCl.

These physical changes closely parallel the development of tension. They are enhanced by previous treatment with ATP and are diminished or abolished by the presence of divalent cations.

To study the swelling of the preparations in water quantitatively, the muscle fibers were tied to a light frame made of a glass capillary, thereby preventing changes in length during the experiment. Preparation together with the frame was weighed on a torsion balance after wiping off fluid with filter paper.

Fig. 5 shows that an isometric contraction produced by ATP, in contrast to that caused by water, does not produce a significant change in weight. Confirming Szent-Györgyi (11) a considerable amount of water (about 30 per cent) is lost from the muscle if the fibers are allowed to shorten in a solution of ATP. Possibly this water is lost for mechanical reasons, being squeezed out by a rise in pressure inside the fibers.

It is important that in the contraction produced by washing out electrolytes, and in contrast to that caused by ATP, only a small amount of tension is recovered after the muscle is allowed to shorten. For this reason, while the tension produced in distilled water is appreciable, the change in length is small. In experiments with an isotonic lever it was found that a preparation under a load of 30 gm. per cm.<sup>2</sup> cross-section shortens by about 5 per cent of its length when electrolytes are removed.

It is certain, however, that the contraction is due to the same contractile elements which are involved in the ATP contraction, not to supporting structures. This is shown by the fact that, if a contraction is produced by ATP and if the muscle is allowed to shorten by one-third of its length or more, the effect of washing out electrolytes is again present at the shorter length.

*Interpretation*

1. *Physicochemical Mechanism.*—A simple explanation of the contraction caused by washing out electrolytes is the following. The charge of the anionic

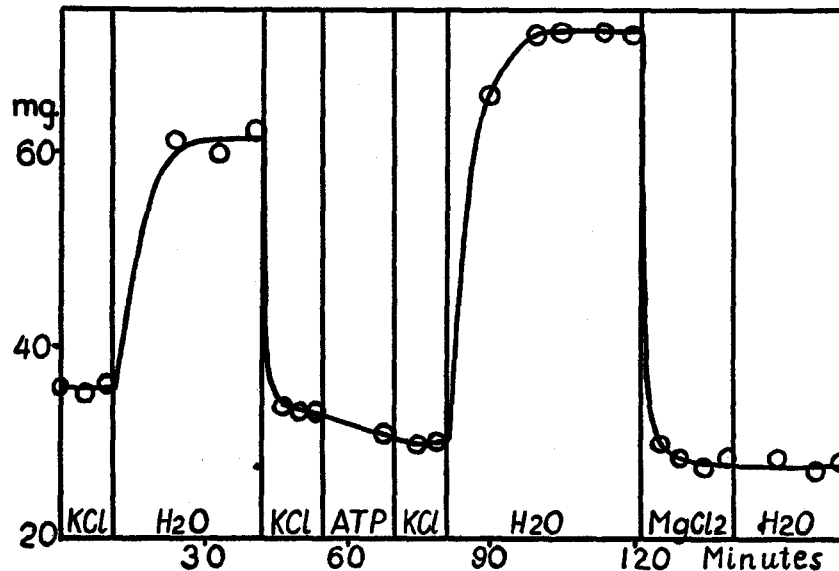


FIG. 5. Weight of a preparation in isotonic KCl and MgCl<sub>2</sub>, ATP 0.2 per cent, and distilled water.

and cationic groups of proteins is neutralized by the accumulation of the oppositely charged ions of the surrounding fluid. Washing out these ions increases the total charge of the protein, causing mutual attraction of oppositely charged groups and shortening of the molecules. That an electrostatic mechanism is involved in these phenomena is confirmed by the great difference in the effects of monovalent and divalent cations.

An alternative explanation of the phenomena just mentioned has been proposed by Monger and Wassermann (6) to explain the contraction of alginate fibers which occurs when calcium ions are replaced by monovalent ions. They assume that calcium ions form salt bridges between adjacent chains. After these bridges are abolished the fibers swell slightly and the chains are able to follow their tendency to curl up.

If this explanation were correct, agents which loosen up intermolecular bonds would also be expected to cause contraction and swelling. However, urea and pyrophosphate, which make extracted muscle fibers very soft and transparent without destroying contractility (1), never cause contraction nor swelling. Distilled water, on the contrary, makes the fibers somewhat more rigid as shown by the fact that, except when tension is very high, the passive decrease of tension after stretch or after an ATP contraction is slightly slower in distilled water than in the presence of electrolytes. These observations cannot be reconciled with the assumption that washing out electrolytes causes a loosening of chemical bonds.

That electrostatic forces can change the length of chain molecules has been demonstrated experimentally. Thus, Staudinger (9) and Fuoss and Strauss (3) have shown that the viscosity of long-chain polyacids decreases with increasing salt concentration, suggesting a shortening of the chains caused by the discharge of the polar groups. Still more directly, Kuhn and Hargatay (4) produced solid oriented films of polyacrylic acid with which they reproduced some of the characteristic responses of muscle and which they consider as models of muscular contraction. These films become longer when immersed in alkali and shorten again on addition of acid. Relaxation in this system is due to the electrostatic repulsion of charged carboxyl groups which dissociate at high pH. Contraction is caused by the discharge of these groups at low pH, which permits the long molecules to assume their statistically more probable shorter length. It is noteworthy that this system, like muscle, swells in the charged, here the relaxed, condition.

In these artificial systems charge of the polar groups causes relaxation and swelling, discharge contraction and shrinking, just the reverse of what has been found for muscle. The difference is probably due to the fact that proteins in contrast to polyacids, contain cationic and anionic groups which attract each other when charged. If, therefore, the system of polyacids can be considered a model of muscle, it appears to work in reverse.

*2. Role of ATP.*—The enhancing effect of ATP indicates that it facilitates the dissociation of cations from the protein of muscle. Because the effect persists long after ATP is washed out, no energy transfer is involved. A simple explanation is the assumption that ATP removes from the preparation some constituent which diminishes the dissociation of cations. This assumption is plausible because ATP not only dissociates actomyosin but also the complex between myosin and glycogen (11) and, therefore, probably also dissociates some other complexes. Indeed ATP solutions, after they have been applied to a muscle preparation, foam readily, showing that some substance has been removed from the muscle.

This explanation of the effect of ATP is in agreement with the following observation. If a preparation is immersed for a few minutes into a 5 per cent

gelatin solution (Eastman purified calf-skin gelatin) and if the gelatin is washed out by 0.16 molar KCl, distilled water has little or no effect on tension, opacity, and weight. ATP subsequently applied causes a strong contraction and restores the effects of washing in distilled water (Fig. 6). The obvious explanation for this observation is the assumption that the contractile proteins adsorb some constituent of gelatin which diminishes the dissociation of bound cations and that ATP dissociates this complex. Not all protein solutions, however, give this effect. Heparinized dog's plasma had no noticeable effect on the preparation.

3. *Physiological Significance.*—That localized variation in the concentration of ions, producing electrostatic effects between charged groups of proteins, may be an important factor in muscular contraction has been pointed out recently by Edsall (2). However, as shown above, the contraction produced by such a mechanism differs considerably from normal contraction. The small

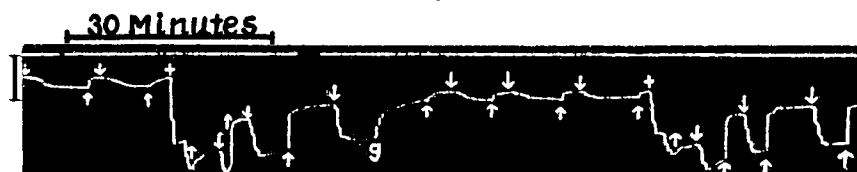


FIG. 6. Effect of 5 per cent gelatin solution (applied at g) on subsequent contractions produced by washing. Isometric contractions as in Fig. 1, At + a 0.25 per cent ATP solution was applied.

degree of shortening produced is particularly disappointing, but not unexpected in view of the short range of electrostatic forces. This mechanism may, nevertheless, be part of normal muscular contraction. In support of this view the effect of ATP, which increases dissociation of bound ions and enhances contraction, may be mentioned. That the phenomena observed reveal something significant on the nature of contractile matter is suggested by the fact that they were observed in all types of muscles studied so far, including cardiac muscle and smooth muscle of several species.

#### SUMMARY

The effects of changes in electrolyte concentration on muscles which had been preserved in 50 per cent glycerol or washed in water were studied. The psoas preparation of Szent-Györgyi was generally used, but smooth and cardiac muscle gave the same results.

If the preparations are immersed in 0.16 molar NaCl or KCl and if the electrolyte subsequently is washed out with distilled water, tension rises. This effect is not obtained if solutions of  $\text{CaCl}_2$  or  $\text{MgCl}_2$  are used, but it is restored by brief immersion in NaCl or KCl solutions. Changes in pH have no effect.

It is concluded that divalent cations are bound more firmly than monovalent ions, but that divalent exchange with monovalent ions.

After the application of ATP washing out electrolytes produces a much larger and more rapid rise in tension. This effect persists after ATP has been washed out and seems to be due to the removal of a substance which diminishes the dissociation of bound cations.

Washing out electrolytes also causes a large increase in transparency and swelling. These effects are also enhanced by previous application of ATP and are abolished or diminished by divalent cations.

The rise in tension and the swelling are explained as the result of an increase in the charge of the polar groups of the proteins. Because this mechanism produces only a small degree of shortening, it does not explain normal contraction, but it may be a part of this process. The significance of the phenomena described in relation to recent theories of the mechanism of muscular contraction is discussed. The observations show that increase in the charge of the contractile proteins causes contraction, not relaxation, as has been commonly assumed.

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