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The Mechanism of Muscular Relaxation

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Relaxation has not been investigated as systematically as contraction. Yet many fundamental problems of muscle physiology cannot be solved without some knowledge of this phenomenon. Before we can explain contraction we have to understand the physico-chemical properties of the muscle proteins at rest. We must know whether liberation of chemical energy is associated with contraction or relaxation. The important question has also been raised whether contraction can occur without relaxation and whether, therefore, in such cases relaxation must be initiated by the nervous system like contraction.

In a discussion of such fundamental questions it is imperative to marshal evidence from many sources. Is is important to consider not only skeletal muscles, however favorable they may be in some respects, but also smooth muscles because they furnished important information on relaxation. An entirely new approach to this problem opened up through the use of simplified systems like extracted muscle fibers. These preparations, which we owe mainly to SZENT-GYÖRGYI[2], made it possible to reconstruct the basic phenomena of muscular activity, including relaxation, with the products of the disintegration of tissues and thereby have given some insight into the nature of relaxation on a molecular level. In this review it will be attempted to synthesize the information which has been gained by these different approaches.

Contraction without Relaxation: The Tonus Problem

It has often been postulated that in some muscles contraction represents a state, called tonus, which is maintained without energy expenditure. In other words, it is assumed that relaxation is not automatically coupled with contraction as it is in skeletal muscle.

The most important argument for such a "catch mechanism" has been the observation that some smooth muscles can remain in a contracted state for long periods of time. However, this observation is not sufficient evidence for the hypothesis because the slow onset of fatigue is adequately explained by the slowness of the response of smooth muscle[1]. Measurements of heat production in isolated smooth muscles have actually shown that maintenance of tension requires continous energy expenditure, but many times less than in striated muscle[2]. The differences between these muscles, therefore, evidently are quantitative. This question has previously been discussed in greater detail[3]. A physico-chemical explanation of the high economy of smooth muscle will be given below.

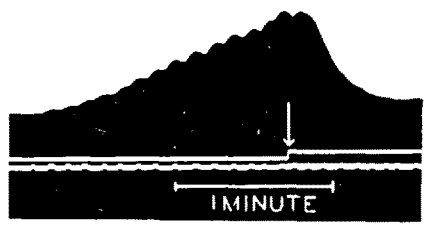

Fig. 1.—Inhibition in muscle of blood vessels of frog's leg. Each electric shock to sympathetic (lower signal) produced a response until blocked by stimulation of dorsal roots (upper signal).

The assumption of a special tonus mechanism can be tested still in another manner. If tonus is contraction without relaxation it must be assumed that relaxation is actively initiated by the nervous system like contraction. In some muscles, particularly smooth muscle, stimulation of certain efferent fibers indeed cause relaxation, an observation which has been frequently mentioned to support the hypothesis of a distinct tonus mechanism. Let us examine, therefore, our present knowledge of the nature of inhibition of smooth muscle by peripheral nerve fibers.

This problem was studied on the blood vessels of the frog's legs[4]. Their responses were recorded by a sensitive flowmeter which measured the flow of perfusion fluid. A sustained contraction of the smooth muscle was elicited by stimulating the sympathetic trunk with single stimuli applied at intervals of a few seconds. If now the vasodilator fibers in the dorsal roots were stimulated, the muscle relaxed completely. Figure 1

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[2] A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction* (Academic Press, New York, 1951).

[1] A. D. RITCHIE, *The Comparative Physiology of Muscle* (Cambridge University Press, London, England, 1928).

[2] E. BOZLER, J. Physiol. *69*, 442 (1930).

[3] E. BOZLER, Exper. *4*, 213 (1948).

[4] E. BOZLER, Am. J. Physiol. *117*, 457 (1936).

shows that this effect was produced because the inhibitory impulses blocked the responses to the vasoconstrictor impulses.

In another type of experiment the effect of the vasodilator impulses was imitated by adding acetylcholine in low concentrations (10^{-7} to 10^{-8}) to the perfusion fluid[1]. This drug also blocked the responses to single shocks but after several stimuli in rapid succession the muscle contracted (Fig. 2). During inhibition, therefore, the muscle can respond, but only after previous facilitation.

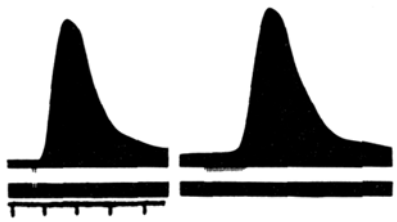

Fig. 2.—Effect of acetylcholine on responses of blood vessels of rabbit's ear. Signal indicates single stimuli to auricular nerve. On right acetylcholine ($2 \cdot 10^{-8}$) was added to perfusion fluid. Time: 10 s.

Experiments on visceral smooth muscles throw additional light on the nature of inhibition. In these muscles excitability is diminished or abolished, during inhibition[2]. However, under normal conditions, inhibition does not stop the initiation of impulses. This is shown by the observation that in the stomach and intestine rhythmic electric activity continues while all visible movements have ceased[3]. Also the amplitude of the action potentials usually is not diminished during inhibition; but their shape is altered strikingly. In the stomach of the dog[4] for instance, the duration of the monophasic potential is reduced from about 6 s to as little as 1 s (Fig. 3). This effect is similar to the shortening of the potential of the auricle produced by vagal stimulation or acetylcholine, but it is much more extreme in smooth than in cardiac muscle. Also in the skeletal muscles of crustaceans the discharge of muscular action potentials may continue during inhibition[5].

These studies show clearly that inhibition causes relaxation by diminishing or suppressing the ability to respond. In no case has a process been demonstrated which actively terminates a state of contraction such as it must be postulated on the assumption that tonus is a state of contraction without energy expenditure.

Recently new arguments have been advanced to support the idea of tonus as a state of contraction without relaxation. It has been reported 'that glycerol extracted muscle fibers stay contracted after a contraction induced by adenosine triphosphate (A.T.P.) even after A.T.P. has been washed out. The opinion was expressed that this represents a state of contraction without relaxation. In the meantime, however, this argument has been invalidated because it has been shown that such preparations do relax, although very slowly, suggesting that maintenance of tension requires the continuous breakdown of A.T.P.[1].

Another argument is based on the observation that in the adductor muscle of Mytilus a strong shock can break up a sustained contraction produced by strong direct currant[2]. However, it is also possible to stop fibrillation of the heart with a strong shock. Surely, no far reaching conclusions can be drawn from such findings without further analysis.

The hypothesis of a tonic contraction without energy expenditure, originally advanced by BETHE and PARNAS, has been very fruitful because it has dramatically emphasized the great differences in the properties of muscles, but it may be questioned whether at present, in the absence of any acceptable evidence, it is still a profitable basis for discussion.

It need not be assumed, however, that all sustained muscular activity consists in a regular discharge of brief impulses. Tetanic contractions are a peculiarity mainly of skeletal muscles. In smooth muscles, states of excitation lasting for many seconds often occur[3].

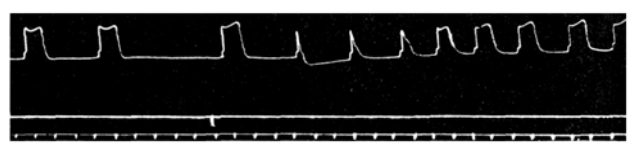

Fig. 3.—Action potentials from stomach of dog. Each wave represents a monophasic potential of one peristaltic wave. At signal 0·03 mg adrenaline was injected intravenously causing shortening of each potential and increased frequency of discharge. Time: 5 s.

Furthermore, there are not only all or none conducted responses but also slow non-propagated contractions which play a role in the initiation of conducted impulses[4] and perhaps in responses elicited by drugs. All these types of activity involve a depolarization of the cell surface and liberation of energy and, therefore, are not basically different from tetanic contractions.

Molecular Mechanism of Relaxation

Role of A.T.P. The state of relaxation is characterized by the softness of the muscle fibers and their readiness to respond to stimuli. What are the physical

[1] E. BOZLER, unpublished.
[2] E. BOZLER, Am. J. Physiol. *130*, 627 (1940).
[3] E. BOZLER, Am. J. Physiol. *146*, 496 (1946).
[4] E. BOZLER, Am. J. Physiol. *144*, 5 (1945).
[5] G. MARMONT and C. A. G. WIERSMA, J. Physiol. *93*, 173 (1938).

[1] E. BOZLER, Am. J. Physiol. *167*, 276 (1951). – H. H. WEBER, Z. Elektrochem. *55*, 511 (1951).
[2] F. R. WINTON, J. Physiol. *88*, 492 (1937). – A. V. HILL, Nature *166*, 415 (1950). – C. A. G. WIERSMA, Ann. Rev. Physiol. *14*, 159 (1952).
[3] E. BOZLER, Am. J. Physiol. *136*, 553 (1942).
[4] E. BOZLER, Exper. *4*, 213 (1948).

and chemical factors producing this state of the contractile elements? To answer this question would be to explain the nature of the living cell, but some significant observations on this problem have been made recently.

When the muscle fibers die, their physical properties change strikingly; they become less extensible, more opaque and, usually, they also shorten slightly. Szent-Györgyi and others have come to the conclusion that this state, called rigor, is caused by the disappearance of A.T.P. This assumption is strikingly confirmed by the observation that under certain conditions muscle fibers can be transformed by A.T.P. from a state of rigor into one closely resembling normal relaxation[1]. Muscle fibers which were washed in water or preserved in glycerol were used in these experiments. Such fibers become translucent and plastic (Fig. 4) in suitable solutions containing A.T.P. and then contract rapidly and vigorously if a small amount of $CaCl_2$ is added. The whole activity cycle of muscle can be closely imitated with such non-living muscle fibers by first bringing about a relaxed state by A.T.P., then eliciting a contraction by Ca-ions, washing out these ions and finally causing relaxation again by A.T.P., as shown in Figure 5. The similarity with the cycle of normal muscle is enhanced by the observation that A.T.P.-ase activity is weak in the relaxed state and increases several fold on addition of Ca-ions[2].

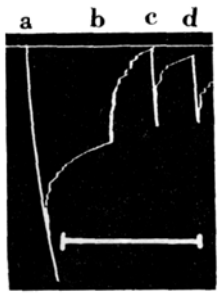

Fig. 4.—Softening action of A.T.P. on extracted muscle fibers. Record of tension (increase downward). At *a*, fibers were stretched 10 per cent; at *b*, fibers in 1 per cent A.T.P., causing rapid drop in tension; at *c*, fibers stretched 50 per cent; at *d*, 30 per cent. Time: 15 min.

The relaxing effect of A.T.P. may seem surprising because previously A.T.P. has always been found to cause contraction. Actually A.T.P. can produce contraction or relaxation depending on certain conditions. Relaxation is obtained only in muscle fibers which have been stored for not more than a few days and only if Mg-ions are present (1 to 10 mM/liter).

How does A.T.P. transform the contractile elements from a state of rigor to one of relaxation? Szent-Györgyi has postulated that in the relaxed state the contractile protein actomyosin is combined with A.T.P. The following observations confirm this assumption. If muscle fibers which had been brought into a relaxed state by A.T.P. are washed with a KCl solution containing Mg-ions, they remain in a relaxed state for some minutes. Although no free A.T.P. is present, the fibers differ strikingly from those in rigor:

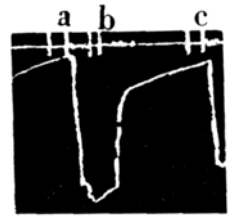

Fig. 5.—Activity cycle of muscle as reproduced in extracted muscle fibers. At *a*, $CaCl_2$ (0·5 mM/liter) caused a contraction in fibers previously brought into a relaxed state by A.T.P. At *b*, fibers were washed with saline. A.T.P. subsequently again caused rapid relaxation. At *c*, $CaCl_2$ caused another contraction.

(1) they remain highly extensible and translucent; (2) they do not respond to A.T.P.; (3) addition of small amounts of Ca-ions (0·1 mM/liter) causes rapid contraction (Fig. 6). These observations show clearly that A.T.P. is still present in the washed fibers, but in chemical combination with the contractile proteins, forming an enzyme-substrate complex. Because contraction, although a weaker one, can also be elicited by removing Mg-ions, it is probable that also this ion takes part in the formation of the A.T.P.-protein complex and is necessary for its stability.

The relaxing effect of A.T.P. is not unexpected in view of previous studies on muscle proteins[1]. Under certain conditions A.T.P. lowers the viscosity of actomyosin solutions, an effect which has been shown to be due to dissociation of this protein into actin and myosin. Szent-Györgyi has considered this effect as analogous to relaxation. His conclusion that actomyosin is in a partially dissociated state during relaxation is in good agreement with the effect of A.T.P. on the mechanical properties of the contractile elements.

Mechanical force causing relaxation. Relaxation reverses the configurational change during contraction. What is the force responsible for this effect?

According to a widely accepted theory contraction is due to the tendency of long oriented molecules to assume a more random configuration. Because in such a mechanism the mechanical work released is furnished by an increase in entropy, the molecular arrangement can be restored only by release of chemical energy during relaxation. It is generally assumed in this theory that relaxation is caused by electrostatic repulsion between charged groups. Kuhn and Hargitay[2] actually devised

[1] E. Bozler, Am. J. Physiol. *168*, 760 (1952).
[2] E. Bozler, unpublished.

[1] A. Szent-Györgyi, *Chemistry of Muscular Contraction* (Academic Press, New York, 1951).
[2] W. Kuhn and B. Hargitay, Exper. *7*, 1 (1951).

a model which imitates muscular contraction and relaxation and works on a mechanism of this type.

The role of electrostatic forces has been tested experimentally as follows[1]. Under physiological conditions the charges of the protein molecules are largely neutralized by ions. Washing out electrolytes, therefore, considerably increases the total charge of the protein. This can be accomplished conveniently in glycerol extracted muscle fibers. It was found that such fibers shorten slightly when they are brought into distilled water, never relax as would be expected if electrostatic repulsion were a factor in relaxation. Furthermore, large changes in pH, which also change the charge of the protein, have no effect on the length of the fibers.

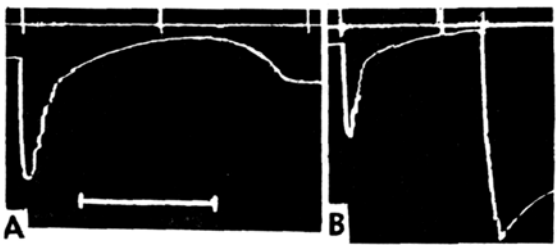

Fig. 6.—Effects of washing out A.T.P. from relaxed, extracted muscle fibers. One per cent A.T.P. caused transient contraction (first signal). After washing out A.T.P. (second signal) tension continued to drop. In *A*, rigor developed later and addition of calcium ions (third signal) had no effect. In *B*, calcium ions (0·5 mM/liter) were added earlier, producing contraction. Time: 15 min.

That relaxation is passive has been demonstrated beyond any doubt by numerous observations. It has been shown, for instance, that relaxing substances not only speed up relaxation but also the isometric loss of tension after passive stretch and that in every case the speeds of these two processes are the same, suggesting that both are due to the same physical forces[2]. Particularly striking is also the fact that rapid relaxation can be brought about reversibly not only by A.T.P. but also by pyrophosphate[2,3] and urea[2]. The last of these substances certainly cannot furnish energy, but is known to loosen up the structure of proteins generally, probably by splitting hydrogen bonds. That breaking up of bonds within the contractile elements is responsible for relaxation is confirmed by the fact that relaxing substances make the fibers very soft and transparent[2].

Studies on normal muscles also support the conclusion that relaxation is passive. In smooth muscles it has been found that the time course of isometric relaxation agrees with that of the loss of tension after stretch in the resting muscle[4] (Fig. 7). In his well known myothermic studies HILL has shown for striated muscle that no heat is produced during relaxation except under condition where mechanical work previously produced is converted into heat within the muscle.

However, in striated muscle relaxation is more complex than in smooth muscle, as shown by the observation, that the physical properties of striated muscle change during relaxation, in contrast to smooth muscle. Extensibility which has been shown[1] to drop suddenly at the beginning of contraction, rises during relaxation and does not return to the resting value in striated muscle until relaxation is complete[2] (Fig. 8). Also, after a quick stretch tension drops in this type of muscle to its equilibrum value much faster than during relaxation. Relaxation of striated muscle, therefore, is not a purely physical process.

No direct information is available on chemical processes during relaxation, but observations on extracted muscle fibers suggest the following explanation. It will be recalled that in these fibers relaxation involves the formation of an A.T.P.-protein complex. Following contraction, during which A.T.P. breaks down, this complex must be restored. It may be assumed that this process, which is possibly due to the presence of free A.T.P., governs the rate of relaxation in striated muscle and causes the rise in extensibility. In smooth muscle relaxation undoubtedly also depends on such a process, but actual relaxation is much slower because a viscous force within the contractile elements opposes extension. The existence of such a force is revealed by a study of the mechanical properties of the relaxed muscle which we shall consider now.

Fig. 7.—Comparison between the drop in tension after extension and after contraction in smooth muscle (retractor of pharynx of snail). In *A*, first isometric contraction (air). Then the muscle was stretched 25 per cent. After isometric loss of tension, another isometric contraction at new length. In *B*, muscle was in 6 per cent CO_2 which increased viscosity and slowed relaxation. Time: 30 s. Note difference in time scale in *A* and *B*.

Mechanical properties of the contractile elements at rest. The mechanical properties of normal striated muscle are largely those of its supporting elements[3]. The study of extracted muscle fibers, however, has furnished important information on our problem. As mentioned before, such fibers, which are in a state of rigor, become very extensible, almost like normal relaxed muscle, after treatment with A.T.P. and other

[1] E. BOZLER, J. Gen. Physiol. *35*, 703 (1952).
[2] E. BOZLER, Am. J. Physiol. *167*, 276 (1951).
[3] H. H. WEBER, Z. Elektrochem. *55*, 511 (1951).
[4] E. BOZLER, Z. vgl. Physiol. *14*, 429 (1931). – E. BOZLER, J. Cell. Comp. Physiol. *18*, 385 (1941).

[1] A. V. HILL, Proc. roy. Soc. London [B] *136*, 211 (1949).
[2] E. BOZLER, J. Cell. Comp. Physiol. *8*, 419 (1936).
[3] E. BOZLER, Biol. Symposia *4*, 260 (1936).

relaxing substances, an effect which certainly must be attributed to the contractile elements themselves.

It is significant that these fibers, after they have been brought into a state resembling relaxation, differ mechanically from normal muscle in one point. A load attached to such a preparation does not cause sudden extension. Instead, after a small, purely elastic, extension the fibers lengthen slowly[1] (Fig. 9). Furthermore, their original length is not restored after unloading. This slow yielding under a load has often been observed in normal, relaxed smooth muscle. It is one of the most impressive and important, but little appreciated, observations on muscle. It shows that the contractile elements are plastic and that resistance of a viscous type opposes lengthening.

Fig. 8.—Extensibility of striated muscle during different phases of relaxation. The curves represent twitches of the retractor penis of turtle. A quick stretch (2 mm) caused a transient rise in tension. The effect decreased during relaxation. Time: 1 s [J. Cell. Comp. Physiol. *8*, 419 (1936)].

What is the cause of this viscous resistance? For smooth muscle it has generally been thought that it is produced by a highly viscous fluid within the muscle fibers. However, the effect of A.T.P. on extracted muscle fibers shows that viscous effects are caused by the contractile proteins themselves. It is unlikely also that these effects are produced by the displacement of the contractile molecules, because extension reverses the configurational changes caused by contraction. Viscous resistance, therefore, probably is intramolecular, caused by weak bonds within the protein molecules which are formed after shortening and which are broken during extension. HILL[2] has described a possible molecular mechanism which accounts for plasticity and other properties of the contractile elements and which helps to visualize the processes under discussion. Figure 10 based on HILL's suggestion, illustrates how extension of the contractile elements can produce viscous resistance. It is assumed that active groups of the contractile proteins, when they come close together during active contraction, form bonds, such as hydrogen bonds, which oppose extension, but are so weak that they can be broken by a small mechanical force. The diagram accounts for the elastic and viscous effects produced by stretching and for the fact that the physical state of the contractile elements and their readiness to respond are not appreciably modified by changes in length. This scheme is merely intended to illustrate the nature of the problem. The plasticity of the contractile elements presents questions which cannot be answered satisfactorily until more is known about the structure of proteins.

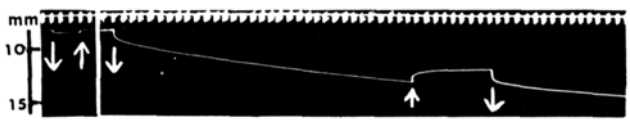

Fig. 9.—Extension of extracted muscle fibers by a load. At ↓ a load of 2 g was applied, at ↑ it was removed. On left preparation was in saline; on right in 0·5 per cent A.T.P. Time: 5 s.

The explanation of viscous resistance presented here at once brings into focus many of the observation mentioned above. In the relaxed state the contractile protein is combined with A.T.P. After the removal of A.T.P. active groups of the protein become free to form intra- and intermolecular bonds. This causes syneresis, shortening, and increased rigidity and opacity, the properties characteristic for rigor. After application of A.T.P., pyrophosphate and urea these bonds become weaker again, but never as weak as in normal muscle, giving rise to "viscous" effects. During storage the strength of the bonds increases further, making relaxation due to A.T.P. progressively slower until finally the relaxing effect of A.T.P. is completely abolished.

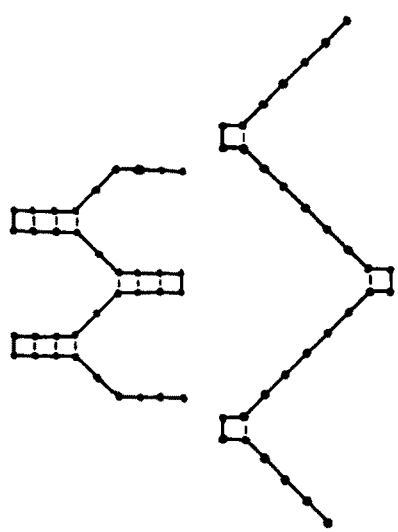

Fig. 10.—A possible molecular mechanism of extension of contractile elements. Black dots represent active groups of protein which form secondary bonds with each other when chains are folded.

Also some properties of normal smooth muscle can be explained on this basis. Because intramolecular bonds which tend to maintain the shortened state of the contractile elements are strong in these muscles, relaxation is slow and the energy expenditure during

[1] E. BOZLER, Am. J. Physiol. (in press).
[2] A. V. HILL, Proc. roy. Soc. London [B] *136*, 420 (1949).

sustained contractions is low. The strength of these bonds may change and is different in different muscles. In the snail's retractor, for instance (not in vertebrate smooth muscle), a manyfold increase is produced by low concentrations of CO_2 or by previous activity, a condition favorable for maintaining tension economically. Physiological differences between different types of muscle, therefore, can be explained as the result of quantitative differences in the properties of the contractile proteins.

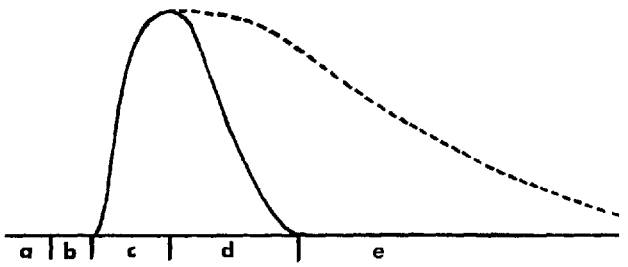

Fig. 11.—Sequence of molecular changes during muscular response in striated muscle (full line), and smooth muscle (broken line). – *a* Relaxed state. Dissociated actin-myosin-A.T.P.-Mg complex. – *b* Latent period. Ca-ions cause formation of actomyosin-A.T.P. complex, decreased extensibility. – *c* Increased breakdown of A.T.P., release of mechanical energy. – *d* Reconstitution of the actin-myosin-A.T.P.-Mg complex and passive extension of contracted elements. – *e* In smooth muscle continued passive extension opposed by viscous resistance.

It remains to fit the processes responsible for relaxation into the remaining part of the activity cycle of muscle. In Figure 11 an attempt has been made to arrange the molecular processes during the response of muscle chronologically. It is assumed with SZENT-GYÖRGYI that at rest actomyosin is combined with A.T.P. and partly dissociated (*a*). At the beginning of contraction Ca-ions cause myosin and action to combine firmly, making the muscle more rigid, perhaps also producing latency relaxation (*b*). This causes increased breakdown of A.T.P. and release of mechanical energy (*c*). During relaxation A.T.P. again combines with actomyosin, causing partial dissociation and increased extensibility. An external force, if present, then can distend the contracted protein chains rapidly (*d*). In striated muscle the last two of these processes go on simultaneously. In smooth muscle, on the other hand, intramolecular bonds resisting passive extension are rather firm and further slow down relaxation (*e*).

Zusammenfassung

Die Annahme eines Kontraktionszustandes ohne Energiebedarf (Tonus) hat keine experimentelle Grundlage. Die durch hemmende Nervenfasern bewirkte Erschlaffung von glatten Muskeln beruht nicht auf einer aktiven Beendigung eines Kontraktionszustandes, sondern auf einer Verminderung der Reizbeantwortung der Muskelfasern. In Gegenwart von Mg-Ionen verwandelt ATP. starre Muskelfasern in einen Zustand ähnlich dem des normalen ruhenden Muskels. Diese Wirkung beruht auf einer chemischen Verbindung des ATP. mit dem kontraktilen Protein. Kleine Mengen von Ca-Ionen steigern die enzymatische Tätigkeit in solchen Fasern und führen eine Kontraktion herbei. Erschlaffung ist passiv, aber in quergestreiften Muskeln wird die Geschwindigkeit der Erschlaffung von einem chemischen Vorgang, wahrscheinlich der Wiederherstellung des ATP.-Proteinkomplexes, beherrscht. In extrahierten Muskelfasern und in normalen glatten Muskeln ist die Erschlaffung ausserdem verzögert durch einen viskosen Widerstand, der durch schwache, intramolekulare Bindungen verursacht wird. Die Stärke dieser Bindungen ist verschieden in verschiedenen Muskeln und bestimmt wichtige physiologische Eigenschaften der glatten Muskeln, besonders die Geschwindigkeit der Erschlaffung und den Energiebedarf während einer Dauerkontraktion.