SOME FACTORS INFLUENCING THE CONTRACTILITY OF A NON-CONDUCTING FIBER PREPARATION*

by

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INTRODUCTION

One of the most important contributions of OTTO MEYERHOF was the discovery of the high energy which may be contained in phosphorylated compounds. Following the description of phosphocreatine (phosphagen) by FISKE AND SUBBAROW[1] and EGGLETON AND EGGLETON[2], MEYERHOF found, in 1927, that the enzymatic decomposition of this compound is connected with the liberation of a large amount of heat[3]. The energy released is about 10000 to 12000 g calories as compared with 2000 to 3000 of other phosphorylated compounds, *e.g.*, hexose mono- and diphosphate, pentose and triose phosphate and other esters, *i.e.*, all those compounds where the phosphate is linked to an alcoholic hydroxyl. MEYERHOF found a similar high energy in argininephosphate which in many invertebrates takes the place of creatinephosphate[4]. A few years later, when in his laboratory, LOHMANN had isolated adenosinetriphosphate (ATP) from muscle, MEYERHOF[5] showed that about 24000 g calories are released by the breakdown of ATP to adenosinemonophosphate (AMP). This is about the same amount of energy for each of the two P as that derived from the P of phosphocreatine. Soon afterwards, two more phosphorylated compounds, intermediates in glycolysis, were found to be rich in energy: phosphoenol pyruvic acid[6] and 1.3-diphosphoglyceric acid, isolated by NEGELEIN AND BROMEL in WARBURG's laboratory[7]. The great significance of MEYERHOF's discoveries of energy-rich phosphates for the understanding of intermediate metabolism and the far reaching implications have been reviewed in this country by LIPMANN[8] and KALCKAR[9].

Among all the energy rich phosphorylated compounds, ATP plays a special rôle. Originally the study of this compound was limited to the glycolytic cycle. More recent studies, however, have shown that ATP has a more general importance, as the source of energy in intermediate cellular reactions, as *e.g.*, acetylation (NACHMANSOHN[10]), urea formation (RATNER[11]) and many others. Although the essential rôle of ATP in intermediate metabolism becomes continuously more evident, its function in the muscle cell in which it was first discovered and studied is still one of the most challenging problems to biologists. From the work of MEYERHOF and his associates, it appeared likely that ATP was involved in the primary changes of the protein during muscular contraction. No other

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U.S. Public Health Service.

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chemical reaction is known to be more closely associated with the contractile mechanism. A new development was initiated in 1939 by ENGELHARDT AND LYUBIMOVA[12, 13] when they tested this idea by studying the interaction between ATP and myosin, which at that time, was the main protein considered to be associated with contraction. Under the stimulus of their observations, the reaction between ATP and the muscle proteins has been extensively studied and considerable progress has been achieved essentially by the work of the NEEDHAMS and SZENT-GYÖRGYI and their associates[14, 15, 16]. The demonstration by STRAUB of a second protein, actin, which combines with myosin to actomyosin, was an important advance in the study of the primary reactions which may underly the contractile process[17].

However, if the interaction of ATP and actomyosin is studied in solution, the element of organization of the protein is not included. Recently, SZENT-GYÖRGYI* has described a muscle fiber preparation which contracts in the presence of ATP. The usual electric stimulus is ineffective. This indicates that the conductive membrane is inactive. In a normal muscle fiber, whether stimulated directly or indirectly, the activation of the conductive membrane which envelopes the muscle cell intervenes between stimulus and contraction. It is only through the activity of this membrane that the contractile process is initiated. Since in the preparation of SZENT-GYÖRGYI the conductive mechanism is excluded but the contractile units are still functioning, as demonstrated by the ATP induced contraction, this fiber offers a most suitable material for the study of factors influencing contraction independent of the action of the conductive membrane. Such a differentiation is of considerable interest for the understanding of some muscular disorders, especially myotonia and familial periodic paralysis. It is with this problem in mind that the present study has been initiated.

MATERIAL AND METHODS

The psoas major of a rabbit was isolated by dissection and then tied at either end to an applicator stick. This preserved the resting length. The muscle was removed in toto by severing its connection at origin and insertion. It was placed in 50% glycerol, kept in the icebox overnight and then stored in 50% glycerol at —10° C. The fibers of the psoas muscle of the rabbit pass throughout the length of the muscle in a parallel fashion. For the present study this muscle appeared suitable but other striated muscles may be used in a similar way.

The main features of the glycerol preserved fibers are: the ease with which a small number of fibers (about 3–10) can be stripped from the main bulk of the muscle; the retention of the structural organization of the fibrils; the modification of the cell membrane to an unexcitable state; and finally the fiber's ability to contract on the addition of ATP.

By grasping the desired amount of muscle fibers in a forceps, they can be peeled from the muscle belly by exerting a slight tension. Forceful pulling on the fibers being detached causes partial interruptions in their continuity which can be noted by holding the fibers to the light and observing regions of increased transparency. Fiber groups 0.5 to 1 mm in diameter were separated from the muscle for study.

The microscopic appearance of the unstained preserved fibers was similar to the normal untreated fibers from the same animal. However, the volume of sarcoplasm was diminished and the diameters of the fibers were decreased appreciably.

The contractions of the unloaded fibers were studied in various experiments. After a number of preliminary observations, the experiments were carried out in the following way. The fibers were suspended in a constant volume of mammalian RINGER'S solution according to KREBS. The contractions were recorded by an isotonic system on a kymograph moving at 3 cm per minute. The suspended fibers were kept in a bath of constant temperature which could be varied according to

* I am greatly indebted to Professor SZENT-GYÖRGYI for the demonstration of this preparation which made this study possible.

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the requirements of the experiment. The standard ATP solution or others tested were added at a rapid and fairly constant rate reaching the suspended fiber almost instantaneously.

Electrical stimulation applied directly to the fibers did not cause contraction. The fibers were inert to supramaximal single and tetanic shocks. On the addition of ATP to the environment of the fibers, a definite and easily recorded contraction developed. As the fibers shortened, their diameters increased. In this respect the contractions resembled isotonic contractions of normal muscle. However, the fibers did not readily relax following the contraction induced by ATP. It was, therefore, necessary to use new fibers for each determination. The stability and constancy of fiber groups became all the more important for this reason. During the first two weeks of preservation the fibers were found to be unstable and variable. On exposure to isotonic solutions of salts, *e.g.*, contained in mammalian RINGER's or saline, pseudo-contractile movements were occasionally observed. After the second week of preservation, more dependence could be placed on the stability of the fibers.

The ATPase activity of the homogenates of the fibers was determined by the method described by DU BOIS AND POTTER[18].

RESULTS

Addition of ATP

Of the compounds tested, ATP and ADP alone elicited contraction of the fibers*. A particularly significant group of substances are listed in Table I. The fiber apparently

TABLE I

COMPOUNDS TESTED TO INDUCE CONTRACTION OF UNLOADED NON-CONDUCTIVE MUSCLE FIBERS (RABBIT). THE ATP AND ADP FIGURES INDICATE THE LOWEST CONCENTRATION WITH WHICH CONTRACTION WAS OBSERVED. THE FIGURES OF THE OTHER COMPOUNDS INDICATE THE HIGHEST CONCENTRATION TESTED

Compound	Concentration (mg/ml)	Contraction
Adenosinetriphosphate (ATP)	0.04	+
Adenosinediphosphate (ADP)	0.5	+
Adenosinemonophosphate (AMP)	100.0	0
Inorganic Pyrophosphate	44.0	0
Acetylcholine	2.0	0
Adrenaline	2.0	0

reacted in a selective manner to ATP and ADP. The threshold concentration of ATP requisite for contraction was less than that of ADP. Moreover, with equimolar solutions of ATP and ADP, the degree of shortening was greater in the case of the former. The amount of shortening of fibers was found to depend on the concentration of the ATP solution employed, approaching a maximum asymptotically (Figs 1, 2).

Unlike myosin threads, the loaded fibers contracted rather than extended in the presence of ATP. Moreover, if the fibers were incapable of shortening because the load was excessive, extension did not occur on the addition of ATP.

Effect of temperature

When the suspended fibers and the added solutions of standard ATP were maintained at 37° C, the extent of shortening was 5.4 times as great as observed under similar conditions at 10° C (Fig. 3). Calculated on this basis there was an increase in the amount of contraction by a factor of 1.9 for each 10° C rise in temperature between 10° and 37° C.

* I am greatly obliged to Dr HARRY G. ALBAUM, Brooklyn College, for supplying adenosinediphosphate and adenosinemonophosphate. The ADP was tested enzymatically by Dr ALBAUM and found by his method to be free of ATP.

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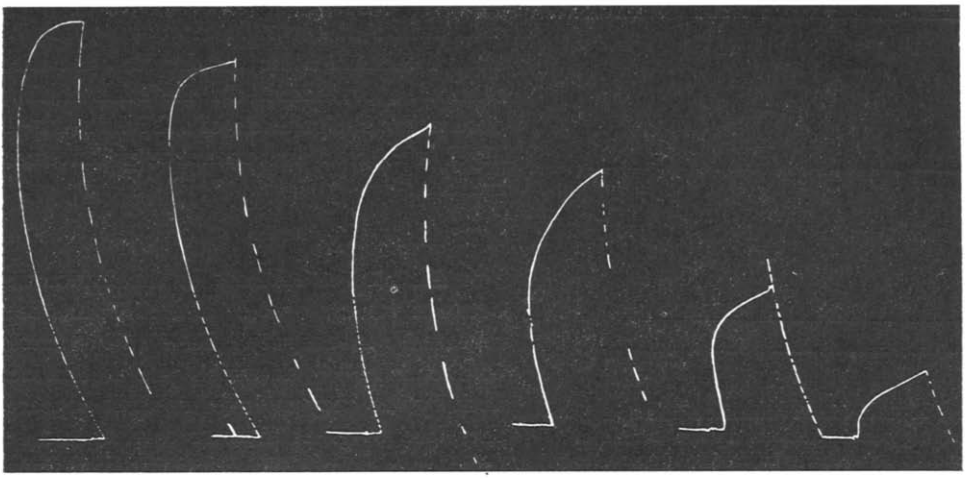

Fig. 1. The series of tracings represent the ATP induced isotonic contraction of fibers 8.5 cm in length recorded on a kymograph moving 3.0 cm per minute. Concentrations of ATP decreasing from 0.04 M in the first to 0.001 M in the last tracing. Magnification 6 ×.

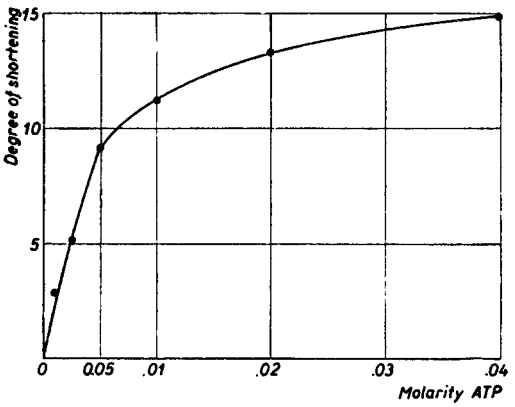

Fig. 2. Degree of shortening of fibers suspended in an isotonic recording system as function of increasing concentrations of ATP. Ordinates: Degree of shortening at a given time in arbitrary units. Abscissae: M ATP concentration. All fibers were of equal length (8.5 cm).

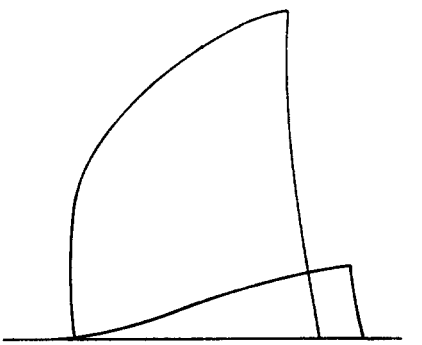

Fig. 3. Effect of temperature on the ATP induced isotonic contraction. Lower curve contraction at 10°C, upper curve at 37° C. ATP concentration 0.002 M.

Effect of p_H

The present experiments were carried out at p_H 7.4–7.6. It was observed that the fibers deteriorated rapidly in solutions beyond the limits of p_H 6.8 and 7.8.

Effect of ions

Sodium ATP caused contraction of the fibers in the absence of other ions. However, magnesium ion activated the reaction of ATP with the contractile proteins of the fibers as shown by the increased extent of shortening in equimolar solutions of ATP (Fig. 4). The optimal concentration of magnesium ion was $1 \cdot 10^{-2}$ M. Potassium in similar concentrations did not manifest the activating effect of magnesium. In the presence of

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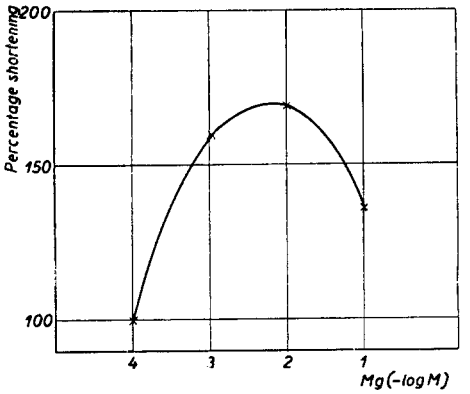

Fig. 4. The effect of magnesium ion in varying concentrations on the extent of isotonic shortening of fibers exposed to 0.002 M sodium ATP. The isotonic shortening caused by sodium ATP alone is arbitrarily assigned as 100%.

calcium ion at $1 \cdot 10^{-2}$ M there was precipitation of the nucleotide and therefore the effect cannot be evaluated. On the basis of these observations a solution of ATP in $1 \cdot 10^{-2}$ M $MgCl_2$ was used as standard to produce contraction.

ATPase activity

The rôle of ATPase in the interaction between muscle protein and ATP has been repeatedly investigated. It is still a matter of discussion[16, 19] at which phase of muscle activity the enzyme is required. It appeared therefore of great interest to determine to what extent the ATPase activity is preserved in the preparation used. Table II shows the rate of decrease of the enzyme activity. The determinations revealed a gradual decline of activity to about 20% of the initial value, at which level the activity appeared to remain stable.

On the addition of ATP enzymatically inactive fibers when loaded remained at resting length and no extension was noted.

TABLE II

ATPASE ACTIVITY OF MUSCLE FIBERS OF RABBIT PRESERVED IN GLYCEROL AT —10° C, TESTED AT 37° C

Time of preservation (days)	μg P/mg/15 min
0 (fresh	25–30
4	15–17
16	10–12
20	6–8
23	6–8
30	6–8

Inhibitors of contraction

Since it is known that ATPase has –SH groups[13], the effect of –SH inhibitors were studied to find reversible inhibitors of the contractile process. Fiber bundles of a diameter of 0.5 mm or less were soaked in solutions of various compounds and then immersed in $1 \cdot 10^{-2}$ M ATP standard. In suitable cases, the fibers after soaking were set up in the isotonic system and quantitative measurements made.

It was apparent that compounds which combined with sulfhydryl groups effectively inhibited contraction of the fibers (Table III). Of these compounds sodium *o*-iodosobenzoate and mapharsen (*meta*-amino-*para*-hydroxyphenylarsinoxide) proved to be reversible inhibitors. The inhibitory effect of mapharsen was reversed by washing the fibers in saline whereas addition of cysteine to saline was required to remove the inhibition produced by *o*-iodosobenzoate. $HgCl_2$ in $1 \cdot 10^{-4}$ M concentration caused irreversible

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TABLE III

INHIBITION OF ATP INDUCED CONTRACTION IN NON-CONDUCTING MUSCLE FIBERS (RABBIT) BY SOME COMPOUNDS REACTING WITH –SH GROUPS. AFTER EXPOSURE THE FIBERS WERE SOAKED IN SALINE CONTAINING 0.01 M CYSTEINE EXCEPT IN THE CASE OF MAPHARSEN IN WHICH SALINE ALONE PROVED TO BE EFFECTIVE

Compound	Concentration (M)	Exposure (min)	Time of washing (min)	Reversibility
o-Iodosobenzoate	$1 \cdot 10^{-3}$	20–25	120	+
o-Iodosobenzoate	$5 \cdot 10^{-4}$	30–40	90	+
Mapharsen	$1 \cdot 10^{-4}$	20	10	+
Mercuric chloride	$1 \cdot 10^{-4}$	20	> 120	—
Mercuric chloride	$1 \cdot 10^{-3}$	9	> 120	—
H_2O_2	$3 \cdot 10^{-1}$	7	> 120	—

inhibition of contraction. At a concentration of $1 \cdot 10^{-5}$ M, however, the inhibitory effect of this compound appeared neglibible.

Other compounds tested and found without an inhibitory effect on the contractile process were sodium monoiodoacetate $1 \cdot 10^{-2}$ M, sodium pyrophosphate $4.4 \cdot 10^{-2}$ M, sodium arsenate $3 \cdot 10^{-2}$ M, sodium arsenite $1 \cdot 10^{-2}$ M and antimony, tartrate and chloride, $8 \cdot 10^{-2}$ M.

Since *o*-iodosobenzoate is a reversible inhibitor of contraction, the following experiments were carried out. Thirty fiber units 6–8 cm in length were placed in a solution of *o*-iodosobenzoate $5 \cdot 10^{-4}$ M in saline. At various intervals 2 cm were cut from some of these fibers, and the sections tested for contractility in a standard ATP solution $1 \cdot 10^{-2}$ M. After 40 minutes, none of the parts of the fibers so tested contracted on exposure to the ATP standard. The fibers were then removed from the inhibiting solution. Ten were placed in saline containing cysteine in $1 \cdot 10^{-2}$ M, the remainder in ATP $1 \cdot 10^{-2}$ M for either 2 or 10 minutes. The experimental groups which did not contract during exposure to ATP were removed from the ATP and washed in saline for 10 minutes and then soaked in saline with $1 \cdot 10^{-2}$ M cysteine for 12 hours. The fibers placed directly in the cysteine saline solution were tested by removing a unit and exposing it to ATP $1 \cdot 10^{-2}$ M. Contractility had returned in 90 minutes. The fibers which were soaked either 2 or 10 minutes in ATP prior to their transfer into the cysteine saline were tested for return of contractility in a manner similar to the former group. During the 12 hours of observation, measurable shortening responses did not appear.

When fibers soaked in *o*-iodosobenzoate $1 \cdot 10^{-3}$ M ceased to contract, they were washed in saline for 10 minutes and homogenized. At that period, the ATPase activity of their homogenates ranged from 3–5 μg P/mg/15 min. A part of the saline washed fibers were then regenerated in a solution of cysteine $1 \cdot 10^{-2}$ M. At the earliest moment when contractility returned, the homogenate revealed an ATPase activity of 5.5–8 μg P/mg/15 min.

Fibers preserved in glycerol and then soaked in cold saline for 10 days retained their ability to contract when their ATPase activity was 6 μg P/mg/15 min or above. Below this level contraction was absent.

Effect of biologically active compounds

Fibers were exposed for 30 to 60 minutes to a number of substances known to have an effect on the contraction of normal muscle. In Table IV are listed the compounds

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and the concentrations used. The degree of shortening on addition of standard ATP was compared with control fibers. To determine the possibility of simultaneous activation of the contractile process, test solutions of adrenaline, acetylcholine and histamine were prepared in ATP standard. These were added to fibers which had been previously soaked in corresponding solutions without ATP. None of the compounds enumerated affected the ATP induced contraction, whether the fibers were exposed to them prior to the contact with ATP or simultaneously.

TABLE IV

COMPOUNDS WHICH HAD NO EFFECT IN THE CONTRACTIONS INDICATED ON THE NON-CONDUCTING MUSCLE FIBER (RABBIT) NOR CHANGED THE ATP INDUCED ISOTONIC CONTRACTION. TIME OF EXPOSURE: 30–60 MIN 24° C

Compound	Concentration mg/mm	Compound	Concentration mgm/mm
Adrenaline	0.002–0.09	Strychnine	0.5
Acetylcholine	1.0 –2.0	Veratrine	0.5
Eserine	0.05 –2.0	Ryanodin	1.0
Prostigmine	0.5 –1.0	Digitoxin	0.2
Caffeine	0.5	Histamine	1.0
DFP	1.0	Quinine	0.4–0.6
		Cocaine	1.0

DISCUSSION

The SZENT-GYÖRGYI preparation may be considered a prototype of the contractile elements of normal muscle. For the study of contraction, it is intermediate between the intact cell and isolated systems (and proteins) in solution. Since the structure of the preserved fibers appears similar to the normal, they probably retain a considerable degree of the orientation and organization of the contractile proteins originally present. Partly for this reason, contraction rather than extension, as seen in the randomly constituted myosin threads, occurs after the addition of ATP to the loaded fibers. Also the supportive action of the sarcolemma mechanically prevents separation of the fibrils' contractile units while they are undergoing spatial rearrangement associated with the process of contraction.

ATP and ADP but not adenylic acid cause contraction of the fibers. Quantitative relationships between concentrations of ATP and ADP and the degree of shortening of the fibers require further investigations. It is apparent, however, that ATP is at least 10 times more effective in causing shortening than an equivalent amount of ADP. Since no enzyme is known to exist in muscle which splits ADP, the effect obtained with ADP may appear surprising. In previous observations reported ADP preparations were not entirely free of significant amounts of ATP and the action of such preparations could be attributed to ATP. The preparation of ADP used in these experiments was free of ATP, as tested enzymatically. However, it is possible that the ADP was converted by myokinase to ATP prior to its action. The fibers have not been examined for the presence of this enzyme.

Under the conditions of the present experiments, contraction of the fibers was not followed by comparable relaxation despite washings in solution containing NaCl, KCl,

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$CaCl_2$ or $MgCl_2$ in various concentrations. Fibers which contracted as little as 20% of initial length were not restored to their original length. Relaxation may be a more complicated process than contraction depending on the integration of several reactions performed poorly, if at all, in this preparation. That ATP induces contraction and not relaxation of the fibers does not indicate at which phase of contraction dephosphorylation of ATP occurs[19].

It has been observed that fibers inhibited from contraction by *o*-iodosobenzoate and then exposed for 2 minutes to ATP did not regain their contractility after prolonged washing in cysteine saline. This may indicate a reaction of ATP with proteins of the fiber possibly independent of that initiating contraction. This observation may offer an explanation for the inability of the fibers to relax, since in the usual experiments performed to measure isotonic contraction, the fibers were exposed to ATP for periods longer than 2 minutes.

It is noteworthy that the contraction of the fibers produced by ATP is enhanced by the addition of magnesium ions. This effect finds its analogy in the action of this ion in increasing the adsorption of ATP by actomyosin[16]. The magnitude of the effect and the optimal concentration of magnesium ion at which it occurs are in harmony with similar observations in isolated ATP-actomyosin systems.

Activation of the fiber contraction by magnesium contrasts to its depressing effect on the intact muscle[20]. Further observations are necessary to decide whether this may indicate that the magnesium effect in the intact fiber is due to an action on the conductive membrane.

Compounds like mapharsen and *o*-iodosobenzoate which reversibly inhibited contraction of the fibers inactivate ATPase activity of myosin[21]. The inhibitors are not specific for ATPase but rather oxidize or combine with thiol groups in general. By measuring the ATPase activity of the homogenates of the fibers, one may secure an index of their efficacy in affecting available –SH groupings. However, the inactivation of ATPase may not be directly correlated with the ability of these inhibitors to prevent contraction. The sulfhydryl groups binding actin to myosin, *e.g.*, are susceptible to effects of these inhibitors[21]. The loss of fiber contractility may be related to a stabilization or blocking of sulfhydryl linkages of the contractile proteins themselves.

By means of the elemental contractile system under study, the action of the biologically important compounds listed in Table IV can be further differentiated. All the substances enumerated are known to affect the process of contraction of intact muscle fibers. Since they are ineffective in influencing fiber contraction produced by ATP, their site of action may be assumed to be elsewhere. From data available it is probable that they affect contraction of intact fibers through their action on the conductive membrane of the muscle either at the neuromuscular junction or along the fibers. Of particular interest in this connection is the absence of any effect of the cholinesterase inhibiting compounds, such as di*iso*propylfluorophosphate and eserine, on the contractile process. This does not support the assumption of a general toxic effect of these compounds as proposed by some investigators, but is consistent with the view which attributes their effect to blocking conduction[22].

The observations presented show the usefulness of the non-conductive contractile preparation of muscle described by SZENT-GYÖRGYI. The system simplifies the study of the contractile process and offers an opportunity to study chemical and pharmacological factors affecting contraction as distinct from conduction.

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I am grateful to Dr DAVID NACHMANSOHN for his suggestions and advice in the conduct of this research.

SUMMARY

A preparation of muscle fibers preserved in glycerol has been described by SZENT-GYÖRGYI, in which the contractile elements remain intact whereas the conductive membrane is not functioning. Properties of such fibers and factors influencing the contractile mechanism independent of conduction have been studied. The following essential results have been obtained.

1. Of a great number of compounds tested, only ATP and ADP induced contraction. The concentration of ADP required was more than ten times higher than that of ATP. Adenylic acid and inorganic pyrophosphate had no effect in high concentrations. The same is true for a great number of compounds like acetylcholine, adrenaline, DFP, eserine and many others which are known to affect the normal muscle fiber preparation.

2. Quantitative evaluations have shown that $4 \cdot 10^{-2}$ M of ATP is close to the optimum to induce the contraction of the non-conducting fiber but concentrations as low as $1 \cdot 10^{-3}$ M had a measurable effect.

3. The extent of shortening increased strongly with temperature, for each 10° C rise between 10° and 37° C by a factor of 1.9.

4. The p_H optimum was found to be between 7.4 and 7.6. The fibers deteriorated rapidly in solutions beyond the limits of 6.8 and 7.8.

5. Magnesium ions activate the reaction of ATP with the contractile proteins. The optimal concentration was $1 \cdot 10^{-2}$ M.

6. The ATPase activity in the fiber preparation declined greatly during the first three weeks to about 20% of the initial value at which level the activity appears to remain stable.

7. The effect of –SH inhibitors has been studied. Two of these compounds, *o*-iodosobenzoate and mapharsen, proved to be reversible inhibitors of the contractile process.

RÉSUMÉ

SZENT-GYÖRGYI a décrit une préparation de fibres musculaires préservées dans le glycérol dans laquelle les éléments contractiles restent intacts tandis que la membrane conductive ne fonctionne pas. Les propriétés de telles fibres et les facteurs qui influencent le mécanisme contractile indépendant de conduction ont été étudiés. Voici les principaux résultats obtenus.

1. Sur un grand nombre de composés étudiés seuls l'ATP et l'ADP induisaient une contraction. La concentration d'ADP requise était plus de dix fois supérieure à celle d'ATP. L'acide adénylique et le pyrophospate inorganique n'avaient pas d'effect à des concentrations élevées. Il en était de même pour un grand nombre de composés tels que l'acétylcholine, l'adrénaline, le FDP, l'ésérine et beaucoup d'autres dont nous savons qu'ils affectent une préparation normale de fibres musculaires.

2. Des évaluations quantitatives nous ont montré qu'une concentration de $4 \cdot 10^{-2}$ M d'ATP est près de l'optimum qui induit la contraction d'une fibre non-conductive; cependant des concentrations aussi faibles que $1 \cdot 10^{-3}$ M produisaient un effet mesurable.

3. Le raccourcissement devenait plus fort lorsque la température augmentait; le facteur était de 1.9 pour toute augmentation de 10° C, dans l'intervalle de 10 et 37° C.

4. Le p_H optimum se trouvait entre 7.4 et 7.6. Le fibres se gâtaient rapidement dans des solutions ayant un p_H inférieur à 6.8 ou supérieur à 7.8.

5. Les ions de magnésium activaient la réaction de l'ATP avec les protéines contractiles. La concentration optima était de $1 \cdot 10^{-2}$ M.

6. L'activité adénosine triphosphatasique diminuait rapidement dans la préparation de fibres jusqu'à environ 20% de sa valeur initiale puis, à ce niveau, elle semblait rester stable.

7. Nous avons étudié également l'effet des inhibiteurs d'–SH; deux de ces composés, l'*o*-iodosobenzoate et le mapharsène sont des inhibiteurs réversibles du processus contractile.

ZUSAMMENFASSUNG

SZENT-GYÖRGYI hat ein in Glycerin konserviertes Muskelpräparat beschrieben, in dem die kontraktilen Elemente intakt bleiben, während die leitende Membrane nicht funktioniert.

Die Eigenschaften solcher Fasern und die Faktoren, welche den von Konduktion unabhängigen Kontraktionsmechanismus beeinflussen, wurden untersucht. Dies sind die wichtigsten Ergebnisse.

1. Von der grossen Anzahl der untersuchten Verbindungen bewirkten nur ATP und ADP eine Kontraktion. Die nötige Konzentration war für ADP zehnmal grösser als für ATP. Adenylsäure und anorganisches Pyrophosphat hatten in hohen Konzentrationen keine Wirkung. Das Gleiche gilt für

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eine grosse Anzahl von Verbindungen, wie Acetylcholin, Adrenalin, DFP, Eserin und viele andere, deren Wirkung auf normale Muskelfaserpräparate bekannt ist.

2. Quantitative Schätzungen haben ergeben, dass das Optimum für die Kontraktion einer nicht leitenden Faser nahe bei $4 \cdot 10^{-2}$ M ATP liegt, aber schon Konzentrationen von $1 \cdot 10^{-3}$ M hatten eine messbare Wirkung.

3. Die Verkürzung wird bei steigender Temperatur grösser; für eine Steigerung von je 10° C zwischen 10 und 37° C beträgt der Faktor 1.9.

4. Wir fanden ein p_H-Optimum zwischen 7.4 und 7.6. Die Fasern verderben rasch in Lösungen deren p_H unter 6.8 oder über 7.8 liegt.

5. Magnesiumionen aktivieren die Reaktion von ATP mit Kontraktions-Proteinen. Die optimale Konzentration betrug $1 \cdot 10^{-2}$ M.

6. Die ATPase-Aktivität des Faserpräparates nimmt während der ersten drei Wochen stark ab und scheint dann bei ungefähr 20% des Anfangwertes konstant zu bleiben.

7. Die Wirkung von –SH-Hemmstoffen wurde untersucht und gefunden, dass zwei von Ihnen, Jodosobenzoat und Mapharsen reversible Inhibitoren des Kontraktionsprozesses darstellen.

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Received April 9th, 1949