MODELS FOR THE STUDY OF THE CONTRACTION OF MUSCLE AND OF CELL PROTOPLASM

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Models for the study of the contraction of muscle and other cellular proteins may be compared to an enzyme system *in vitro*. The active protein is isolated and the substrate and the necessary cofactors are added. The simplest models consist of the pure contractile protein, adenosinetriphosphate (ATP), magnesium ion and other ions. So far they have been prepared from muscle only. Muscle shortens and does mechanical work. In order to investigate the underlying mechanism, the active protein of the *in vitro* system must be present in a structure which exhibits the mechanical properties of contracting living muscle.

More complicated models are obtained by extracting, with glycerol and water, muscle bundles, single muscle fibres, or cells taken from tissue cultures. In addition to the contractile protein, these models contain other insoluble proteins. They differ from the living cell, or the living muscle fibre, because the membranes have been destroyed and the fluid of the tissue has been replaced, first by water and glycerol (during the extraction), and finally by pure saline. Owing to the destruction of the membranes, processes of excitation and osmosis do not take place. In addition, the removal of the physiological fluid makes changes of surface tension impossible. Finally, the removal of the crystalloids and of many enzymes may render the models unable to metabolize substances other than those which are added.

Cells of tissue cultures extracted with water and glycerol during the interphase contract to spheres on addition of ATP if they had assumed an elongated shape during their growth. When the extraction took place during the appropriate phase of the growth of the cell, the addition of ATP causes the contraction of the cell equator which marks the cleavage of the telophase. In addition to these contractions of the cell plasma, ATP initiates quite different cell movements, *i.e.*, the elongation of the whole cell and that of the spindle apparatus during the anaphase. Movements of active extension will not be described in this review.

The muscle models prepared from purified actomyosin, as well as fibres and fibrils extracted with glycerol-water, show contraction and relaxation but no electric phenomena.

I. COMPARISON OF MODELS WITH LIVING SYSTEMS

1. Models for the study of muscular contraction. The simplest model showing anisodiametric shortening and development of tension is the oriented actomyosin thread in the presence of ATP. The actomyosin thread was first introduced

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by H. H. Weber (120, 121), who concluded that the mechanical behaviour of resting muscle and its X-ray diagram and birefringence are due to its content of myosin.

Since 1939, interactions between actomyosin and ATP have been discovered starting with the finding of Engelhardt and Ljubimowa (30, 32) that myosin splits ATP and that myosin threads become more extensible by addition of ATP. In 1942 Szent Györgyi (99) discovered that actomyosin threads shrink considerably on the addition of ATP. Thereupon threads which shortened anisodiametrically, were prepared by different techniques (25, 38). However, with these models no development of tension could be studied. Actually, Buchthal's threads lengthened considerably when they were loaded.

Portzehl and Weber (87, 88, 122) first succeeded in making threads from purified actomyosin which also developed tension (300 g/cm^2). Their procedure consists of two steps: first, the protein concentration of the thread is increased to a calculated endpoint. This is done by soaking the threads with a mixture of glycerol and water and by drying them afterwards at a temperature of about 0°C. Second, the protein particles are aligned by slowly stretching the thread. These threads shorten to 30 per cent of the original length the birefringence decreasing with shortening. When suddenly released to an extent of 5 per cent during an isometric contraction, the tension drops to zero and redevelops afterwards (quick release phenomenon of Gasser and Hill (37)). Another type of structure prepared from purified actomyosin which is able to do work on addition of ATP was developed by Hayashi (54), who spread actomyosin into monolayers and afterwards compressed them into thin strands.

These thread models indicate that the contraction of a more complex type of model, developed meanwhile by Szent Györgyi and Varga (101, 113, 114), was due to the reaction between ATP and actomyosin. This latter model consists of fibre bundles of skeletal muscle which have been extracted in glycerol-water mixtures in order to remove salts and water soluble proteins. These bundles were isolated from the muscles of various animals while most of the experiments were performed on extracted bundles from the rabbit psoas. When single fibres were used instead of bundles (117, 119) many phenomena of the contraction of living muscle could be demonstrated in a quantitative manner. Hence, Szent Györgyi's claim (101) that the contraction of the fibre models is based on the same fundamental process as muscular contraction has gained a high degree of probability.

The glycerol-extracted fibres have not been analysed with respect to their protein content. From studies on the extractibility of tropomyosin from muscle tissue and from the protein composition of myofibrils it must be concluded that they contain at least tropomyosin and the so-called stroma proteins, in addition to actomyosin (3, 86). Myokinase, which is present in myofibrils (85), and is removed from actomyosin only by frequent reprecipitations (2, 30, 82), and adenylic acid deaminase, which is so closely associated with myosin that it has been claimed to be identical with myosin (30, 58), are present in glycerol extracted fibres (118), as well as creatinephosphokinase (23, 41, 74). The glycerol-extracted fibre no longer retains a functioning membrane. However, on the basis

of results obtained with the thread model, it seems justifiable to attribute the behaviour of the extracted fibre to the reaction between actomyosin, in its intact structure, and ATP.

The glycerol-extracted single fibre in the presence of ATP develops a tension as high as the maximal tension of the corresponding living muscle (123, see table 1). The extent of shortening, however, is greater than in any living skeletal muscle and is the same for extracted fibres prepared from smooth muscles. Single living fibres of skeletal muscle can be made to shorten to an equally great extent but their behaviour then changes to that of the delta state (93, 94). The living fibre always passes into the delta state if it shortens to less than ⅔ of the original length after an appropriate and intensive stimulation. When the living fibre in the delta state is reextended to its original length, the tensions developed on stimulation are lower and the length-tension diagram differs from the original.

The speed of shortening of extracted fibres is considerably lower than that of living muscle (89, 110). The extracted myofibrils of the rabbit psoas at 20°C. and 80 per cent standard length have a rate of shortening of about 150 per cent/sec. of the standard length during the first one-tenth of a second (89). This velocity is similar to that of a frog sartorius at standard length at 0°C. (59). The rate at 20°C. would be about 4 to 5 times greater; that is, the velocity, though less, is nevertheless of the same order of magnitude. The dependence of the speed of shortening on the load was studied on the glycerol-extracted fibres of the rabbit psoas and of the yellow adductor of *Anodonta coelensis* (smooth muscle). Hill's equation in the modification for lengths below standard (1, 59) was found to apply to the rabbit psoas model if the values for the constants were changed (112). The equation only roughly describes the relationship between tension and velocity of the *Anodonta* models at standard length. It cannot be used at lengths below standard (112). However, it has not been shown whether or not the equation is valid for lengths of the living adductor below standard length. The maximum power of contraction of the *Anodonta* model, as well as of the psoas model, was developed under a load of 0.3 maximal isometric tension. This is in accordance with the living muscle (60, 111). During the shortening the model liberates energy by dephosphorylating ATP. Thus it is possible to calculate the efficiency as a ratio of rate of work to rate of splitting. The maximal efficiency of the psoas model was 25 to 40 per cent at 0°C. which is slightly above that of living frog muscle in spite of its much lower speed of shortening. This is possible because the rate of ATP splitting is also lower than the rate of energy supply in living muscle. The intrinsic and the form birefringence change with length in the same way as they do in living muscle (98).

The difference in behaviour between the actomyosin threads and the glycerol-extracted fibres is probably based on a difference in structure. In the fibre actomyosin is probably left close to the original structure while in the thread the protein particles are less well aligned and the cohesional forces are smaller. In addition, in order to obtain a parallel arrangement of the protein chains, the thread must be subjected to special treatment which might change the native

state of the protein. On the other hand, the thread model has the advantage of greater simplicity compared to the extracted fibre and its further modification, the extracted myofibril, as introduced by Perry (85) and Hasselbach (46).

The third type of model, the structureless actomyosin gel, which shows only superprecipitation, is the most convenient and, in spite of its limitations, is often sufficient for studies of the reactions which influence the interaction between actomyosin and ATP.

The studies of the behaviour of actomyosin in solution on addition of ATP have yielded many interesting results. In solution actomyosin dephosphorylates ATP (2, 5, 32, 102, 103) and its native configuration is affected strongly by ATP, as demonstrated by measurements of viscosity (26, 79, 101, 102, 103), light scattering (15, 67, 68) and birefringence of flow (26, 83). In solution, however, the optimal conditions for the enzymatic activity of actomyosin are quite different from those in the gel and no reaction has yet been found which could be considered to be identical with the elementary process underlying the contraction in a gel. Therefore, only the results obtained from the investigation of the viscosity response or the light scattering will be used below as additional evidence.

The contraction of actomyosin systems is produced only by ATP and ITP (97). The contraction is not followed by subsequent relaxation.

When the thread and the glycerol-extracted fibre in a saline solution up to 0.15 ionic strength are compared to living muscle with regard to extensibility, they resemble a muscle in rigor rather than a resting muscle. Their modulus of elasticity is high (4000 to 7000 gm./cm^2.) and the extent to which they can be stretched without breaking is rather limited (87). As found by Erdös (34), Bate-Smith (7) and Bendall (8), rigor, with or without contracture, occurs in a muscle when most of the ATP is exhausted. In resting living muscle a high concentration of ATP is present, while the rate of energy liberation is low.

Starting from the idea that the plasticizing effect of ATP is due to its polyphosphate group and that ATP keeps the muscle in a resting state if its splitting is prevented, Portzehl and H. H. Weber first succeeded in producing a resting state in an uncontracted, and relaxation in a contracted, model. This effect was, however, elicited by such unphysiological means as adding the sulfhydryl-binding reagent salyrgan (mersalyl, sodium o-[(3-hydroxymercuri-2-methoxypropyl)carbamyl]phenoxyacetate) to inhibit ATPase activity, or replacing ATP with other polyphosphates which are not split by actomyosin. A more physiological relaxation in the models was brought about by a factor discovered by Marsh (76, 77) and first investigated in its effect on contracted fibres by Bendall (9, 10, 11). It is present in a fresh aqueous extract of muscle and its addition to models contracted in the presence of physiological concentrations of ATP reduces the tension to nearly zero. At the same time it inhibits the dephosphorylation of ATP (11, 13, 53). It is probably responsible for the relaxation which Bozler (21, 22, 24) produced by adding ATP in high concentrations to fibre models which had been extracted only briefly. Later, Bozler (23) discovered that the effective concentrations of ATP could be considerably lowered if creatine phosphate was added. Goodall and A. G. Szent Györgyi (41) found

a relaxing effect of creatine phosphate in presence of ATP at pH 5.7. Lorand showed that this effect was probably due to the combined action of ATP, creatine phosphate and creatinephosphokinase which produced a relaxation of the extracted fibre at pH 6.3 (74). Bendall (13) found that the "factor" activity of an extract closely parallels its myokinase activity. Myokinase, as well as creatinephosphokinase, has a relaxing effect in the absence of Ca^{++} and in the presence of Mg^{++}. Under the conditions under which myokinase produces relaxation, it inhibits the rate of liberation of inorganic phosphate from ATP.

When the model is in the resting state, it has a similarly low modulus of elasticity as the living muscle (88). Relaxation is indicated in living muscle, as well as in the models, by the decrease of an isometric tension to zero, or, by the extension of a shortened muscle or model to the original length. However, under the above conditions, the tension of the models is sometimes only markedly reduced without becoming zero. If a living muscle, or a model, has shortened isotonically and relaxation has occurred, very small forces suffice to extend it again to standard length, although, as A. V. Hill (61) has found for living muscle, and as is true of the muscle models, no spontaneous lengthening occurs. However, with respect to single fibres of living muscle and cell models during the second part of the anaphase, evidence of spontaneous lengthening can be found in the literature (65, 93, 94). There is no evidence that relaxation in living muscle must be coupled with an energy liberating process because no heat is released (61). Moreover, work done on muscle during this phase is not absorbed but appears as heat (61). Since the muscle models relax if the splitting of ATP is prevented by salyrgan or if the ATP is removed from a solution containing inorganic polyphosphates, it seems justified to assume that no energy liberating process is involved (124, 125).

2. Models for the study of the contraction of cell protoplasm. Hoffmann-Berling (63, 66) introduced the models for the study of the contraction of cell protoplasm. These are glycerol-extracted cells of tissue cultures. The living cells have an elongated shape during the interphase and when they are extracted in this state they contract to a sphere on addition of ATP. If the cells were extracted after the completion of the anaphase ATP initiates the cleavage of the cell models (65). It is apparently an equatorial contraction of the cell plasma (see 65, fig. 1). In cells extracted after the completion of the early anaphase the addition of ATP causes the chromosomes to continue their separation occasionally beyond the physiological distance.

II. SUBSTANCES AND ACTIVE GROUPS OF THE PROTEIN PARTICIPATING IN THE MOLECULAR MECHANISM OF CONTRACTION

Although the contraction of actomyosin by ATP is markedly influenced by a number of substances, very little is known of their effect on the molecular mechanism. This is in part due to our paucity of knowledge concerning the reaction between actomyosin and ATP itself. It is known that the triphosphate grouping of ATP is essential for the reaction because ADP does not bring about contraction. It is also obvious that the polyphosphate grouping alone is not responsible

since inorganic triphosphate and pyrophosphate do not produce contraction. Among the inorganic polyphosphates only triphosphate is split although much more slowly than ATP. The amino group of the purine does not seem to be involved directly in contraction and dephosphorylation because ITP appears to react with actomyosin in much the same way as ATP. ITP, however, requires much higher concentrations of Mg^{++}, *i.e.*, the amino group appears to lower the Mg^{++} requirement. Studies with thiol-binding reagents indicate that the presence in the protein of certain SH-groups is necessary. The indispensability of Mg^{++} seems to indicate that only the undissociated actomyosin complex is able to contract, since it is only in this form that it is activated by Mg^{++}.

The generally accepted view that the contractile protein must contain actin, as well as L-myosin, has recently been challenged by Engelhardt (31) who prepared, from L-myosin, threads which contracted and developed tension. As this work has not yet been confirmed, we shall restrict ourselves to the actomyosin system. On the basis of the available evidence the following conclusions can be drawn. The contraction of actomyosin depends on the presence of ATP or ITP and of Mg^{++}, on the existence of certain groups such as SH in the protein, and on the ionic strength. The influence of all the salts changes markedly with concentration, so that an ion which activates at low concentrations may inhibit at higher concentrations. All of the salts show a strong interdependence, *i.e.*, the optimal concentration of each is dependent upon the concentration of the others. The evidence secured with cell models suggests that the reaction producing the different kinds of contraction of the cell plasma is similar to that of the muscle models. The contraction of the cell plasma depends on the presence of ATP or of ITP, Mg^{++}, and free SH-groups in the protein, in the same way as the contraction of the muscle models.

III. INFLUENCE OF CHANGES IN THE ACTIVE CONCENTRATIONS OF THE COMPONENTS OF THE SYSTEM

1. Adenosinetriphosphate and related compounds. a) Adenosinetriphosphate. The action of ATP on actomyosin changes greatly with the concentration of ATP. At higher concentrations of ATP substrate inhibition occurs, *i.e.*, the rate of dephosphorylation of ATP is decreased and lower isometric tensions are developed. At very high concentrations of ATP actomyosin is dissolved.

If the effect of different ATP concentrations on the contraction of the models is investigated, one has to take into account the difference in the concentration of ATP inside the model from that in the bath. That inside the models depends on the balance between the diffusion and the breakdown of ATP. With a given ATP concentration of the bath, the radius of a cylinder in which the ATP concentration reaches zero in the center (Grenzschichtdicke) can be calculated according to Meyerhof (78): $r = 2 \times \sqrt{\frac{c_0 \times D}{A}}$; ($r$ = Grenzschichtradius, c_0 = bath concentration, A = rate of decomposition, and D = diffusion constant). The diffusion constant of ATP inside the fibre model is 100 times smaller than in a saline solution (46). Thus, a fibre model with a radius greater than 10μ at

20°C. and an ATP concentration of 0.005 M will have a core of myofibrils which do not contain any ATP. In order to have practically the same concentration inside the fibre as in the bath at 20°C. and 0.005 M ATP, the radius of the fibre must not be greater than 3μ, and at 0.0005 M it has to be about 1μ. These considerations apply not only to fibre models or thread models but to actomyosin particles during precipitation, because superprecipitation results in the aggregation of small particles to larger ones.

Consequently, a curve showing the dependence of the activity of the contractile protein upon the ATP concentration of the solution will, with different kinds of models, vary according to the protein concentration and the thickness of the models. Thus, with increasing ATP concentration, the rate of shortening of myofibrils, or, the rate of splitting of ATP, shows a plateau of constant activity before substrate inhibition leads to a decline in activity (46, 89). The maximal tension and the enzymatic activity developed by fibre models, however, show a typical optimum dependent upon the ATP concentration; the tension and the rate of splitting rise to a peak and then decrease again (56, 119). This sharp maximum characteristic of fibre models is due to their greater thickness, which prevents the penetration of ATP to the center, except at the highest concentrations, approaching values beyond the optimum.

The range of low concentrations of ATP (below 0.0005 M) has been investigated only for the ATPase activity of actomyosin solutions, and not for the models which are gels (84, 106, 116).

The position of the point at which the ATP concentration starts to be super-optimal depends on a variety of factors such as the ionic strength, Mg^{++} concentration, protein concentration[2], and temperature (47, 100, 118a, 119). With myofibrils, the concentration of ATP at which the substrate inhibition begins with respect to the rate of dephosphorylation falls, other conditions being equal, from 0.01 M to 0.03 M ATP when the temperature is lowered from 20 to 0°C. The substrate inhibition begins at lower levels of ATP when the Mg^{++} concentration or the ionic strength are increased (47, 48). Further increase of the ATP concentration results in a partial, and, ultimately, in a complete dissolution of the protein. However, the decrease in contraction is not due to the dissolution of part of the protein. Under certain definite super-optimal conditions, the protein is no longer able to contract without having dissolved (33, 52).

The range of concentration of ATP which is beyond the optimum for the contraction of glycerol-extracted fibres can be drastically shifted by the factors of relaxation. For example, in the presence of the Marsh-Bendall factor, about 0.001 M ATP is, as Hasselbach has shown (53, see fig. 1, 2), already superoptimal.

At high concentrations of ATP (0.03 M), cell models contract to a lesser extent than at optimal concentrations (64). At these high concentrations, the extent of shortening of extracted myofibrils of skeletal muscle is decreased.[3] There have not yet been experiments on the splitting of ATP by the cell models.

[2] In dealing with myofibril suspensions it is not the overall protein concentration which is essential but the concentration inside the myofibrils.

[3] This value for the concentration of ATP at which, with respect to the extent of shorten-

b) *Inosinetriphosphate.* ITP appears to have the same effect as ATP, not only on the solutions of actomyosin and myosin (26, 70, 83), but, as has been shown by Spicer and Bowen (97), also on the actomyosin gel, provided the Mg^{++} concentration is greater than 0.0001 M. Under similar conditions, ITP initiates the contraction of the cell models. The concentrations of ITP superoptimal with regard to the contraction of the cell models are the same as those for ATP (64). No corresponding data exist for ITP with respect to the contraction of muscle models in the absence of relaxing factors. In the presence of the Marsh-Bendall factor, high concentrations of ITP, in contrast to ATP, did not produce relaxation (13).

c) *Adenosinediphosphate and adenosinemonophosphate.* Adenosinediphosphate (ADP) (100, 117) and adenosinemonophosphate (AMP) (99) alone do not induce contraction, nor is ADP dephosphorylated by actomyosin (2, 32, 83). In the presence of an energy-rich phosphate compound and a transphosphorylating enzyme, both compounds can be phosphorylated to ATP which then induces contraction. Thus glycerol-extracted fibres contract on addition of ADP since they contain myokinase (71), which converts ADP to ATP and AMP (69). Furthermore, extracted muscle fibres were found to contract with a mixture of AMP and creatine phosphate (23) but not with creatine phosphate alone. Myokinase, in the presence of traces of ATP, transphosphorylates AMP to ADP (29) which is phosphorylated to ATP by creatinephosphokinase. In concentrations equal to that of ATP, ADP and AMP have not been found to be competitive inhibitors, either for enzyme activity, or for contraction (48, 84).

d) *Inorganic polyphosphates.* As mentioned previously, no inorganic polyphosphate produces contraction. With the exception of triphosphate (70), inorganic polyphosphates are not split by actomyosin. However, the rate of dephosphorylation of triphosphate is very low (107). Due to the similarity in structure, the polyphosphates might be expected to compete with ATP for the same sites on the protein, thereby decreasing the number of ATP molecules combining with actomyosin. Such a competitive effect seems to exist in actomyosin solutions. The dephosphorylation of ATP is inhibited by pyrophosphate to a degree proportional to the ratio of pyrophosphate to ATP (106). Similarly, the rate of ATP splitting is reduced in the presence of inorganic triphosphate (70). Another indication that the polyphosphates combine with the same sites on the protein as ATP is their effect upon the viscosity and light scattering of actomyosin sols. Triphosphate and pyrophosphate cause a similar drop in the viscosity as ATP, provided the Mg^{++} concentration is high enough (0.01 M) (1a, 79). Studies

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ing of myofibrils, the substrate inhibition begins does not agree with the corresponding values of ATP concentration for the dephosphorylation of ATP. However, the assumption that the energy for the contraction is provided by the energy of the dephosphorylation of ATP connotes a close relationship between the maximal rate of work and the rate of splitting of ATP. Such a relationship has been found with regard to the influence of temperature on both the dephosphorylation of ATP and the rate of maximal work (110). The extent of shortening, however, is not determined by the velocity of the enzymatic reaction; the same length might be reached slowly or rapidly.

with pyrophosphate showed that it also produces a decrease in light scattering (106).

The relaxation experiments of Portzehl and H. H. Weber (88, 122) indicate that the polyphosphates also combine with the actomyosin gel. They increase the extensibility of extracted fibres, and contracted fibres relax if the ATP is washed out with 0.02 M pyrophosphate or triphosphate. However, in the presence of a Mg^{++} concentration lower than 0.001 M, the polyphosphates appear to be displaced from the actomyosin by ATP; the isometric tension produced by ATP in the presence of pyrophosphate is as high as in its absence. If no Mg^{++} at all has been added to the solution, no pyrophosphate seems to be bound even in the absence of ATP, for under these conditions no relaxation can be obtained (12).

If, however, the Mg^{++} concentration is greater than 0.001 M, the addition of pyrophosphate, in a concentration as low as 0.004 M, to an ATP solution of twice this concentration leads to an immediate relaxation. This effect, described by Bendall (12), is, however, not due to a displacement of ATP, for pyrophosphate alone in such low concentrations gives only a very slight relaxation without ATP. Under these conditions, pyrophosphate causes an inhibition of 75 per cent of the rate of dephosphorylation of ATP (13). In the presence of ATP and high concentrations of Mg^{++}, the relaxation due to pyrophosphate is markedly influenced by small amounts of Ca^{++} (12). A concentration of 0.0002 M Ca^{++}, which is much too low to diminish the concentration of free polyphosphate or ATP, completely prevents the relaxing effect of pyrophosphate in the presence of ATP or during the inhibition of the dephosphorylation of ATP. In this respect, pyrophosphate shows a behaviour very similar to that of the relaxing factors.

At higher concentrations, especially when the pH is slightly above 7, pyrophosphate and triphosphate change actomyosin irreversibly. The range of concentrations at which this effect occurs appears to be different for the models from different muscles. For the rabbit psoas, A. G. Szent Györgyi (104) found it to occur only above 0.05 M, while for the *Anodonta* model, G. and M. Ulbrecht (109) found a partially irreversible abolition of tension at 0.01 M pyrophosphate. The fibre models of the rabbit psoas, after pretreatment with 0.05 M pyrophosphate, develop only very small tensions and are able to shorten only in a small range of salt concentrations (104).

e) Orthophosphate. Orthophosphate has no influence on the contraction of the models and does not increase their extensibility (88, 117).

2. Magnesium ion and other divalent ions and metal chelating reagents. a) Magnesium ion. Mg^{++} appears to play a very specific role in the reaction between ATP and the contractile proteins both of cell and muscle models. According to Bowen (19) actomyosin seems to have a relatively large amount of strongly bound Mg^{++}. Portzehl (89) pretreated fibre models with ethylenediaminetetraacetic acid (Versene, EDTA) and found the contraction strikingly reduced, the velocity of shortening decreased to a fraction of 1 per cent, while the maximal tension and the extent of shortening fell to 15 to 30 per cent of the respective

original values. It was not determined whether the remaining activity was due to traces of Mg^{++} still present. After the addition of Mg^{++} the contraction increased to the original value (90). Hoffmann-Berling (64) found that the cell models contracted to a greater extent if free Mg^{++} was present in the solution. In the presence of EDTA, the contraction of the cell models was completely suppressed but the inhibition was reversed on the addition of Mg^{++}.

The rate of splitting of ATP by actomyosin treated with EDTA or hexametaphosphate is also reduced to a fraction of the original value. Addition of Mg^{++}, as well as Ca^{++}, restores the rate of splitting (20, 47).

The dependence of the contraction on the Mg^{++} concentration was investigated by means of superprecipitation (47, 101). The superprecipitation increases with increasing Mg^{++} concentration to an optimum around 0.0005 M to 0.005 M, depending on the concentration of ATP, ionic strength, and protein. The optimal concentration of Mg^{++} for the rate of dephosphorylation of ATP and for superprecipitation is in the same range (5, 47, 85). Above the optimal concentration of Mg^{++}, the inhibition increases with the concentration. Mg^{++}, however, has no specific influence on the dissolution of actomyosin. The concentration of ATP, as well as the ionic strength required for the transformation of actomyosin from a gel to a sol, remains unaltered in the presence of increasing concentrations of Mg^{++} (52).

The main difference in the behaviour of ATP and ITP towards actomyosin manifests itself in their different requirement of Mg^{++} (see p. 104). The amount of Mg^{++} which is present in actomyosin, or in the cell models, is sufficient for ATP, but not for ITP, to bring about the superprecipitation of actomyosin or the contraction of extracted cells. The addition of Mg^{++} is necessary for an ITP-induced contraction. The optimal concentration of Mg^{++} is 0.01 M (97).

For the relaxing effect of ATP in the presence of relaxing factors, the Mg^{++} concentration must be above 0.001 M. This holds for the Marsh-Bendall factor (11), Bendall's myokinase extract (13), the Bozler effect with high concentrations of ATP (22), as well as with creatine phosphate and low concentrations of ATP (23), and the creatine phosphate effect at low pH (41). It also holds for the effect of pyrophosphate in the presence of ATP (12). In the absence of ATP, pyrophosphate in high concentrations produces relaxation even at Mg^{++} concentrations below 0.001 M but not if no Mg^{++} is added (12).

b) Calcium ion. Ca^{++} can replace Mg^{++} only as an activator for the dephosphorylation of ATP by the gel. In all other respects it acts as an antagonist to Mg^{++}.

The inhibition of the enzymatic activity of actomyosin in consequence of its treatment with hexametaphosphate or EDTA can be restored, not only by the addition of Mg^{++} but also by that of Ca^{++} (47, 51). The concentration of Ca^{++} required to produce the maximal rate of splitting of ATP is higher (0.01–0.05 M) than that of Mg^{++} (0.0005 M) (5, 47, 85). A decrease in the activity of actomyosin is found only at high concentrations of Ca^{++} (above 0.04 M). The value of this concentration depends on the pH (5, 81, 85).

If, however, the contractility has been abolished by the removal of Mg^{++}, it

cannot be restored by Ca^{++} (89). Furthermore, it should be mentioned that after the removal of alkaline earth ions an actomyosin solution will show the characteristic decrease in viscosity due to ATP only if Mg^{++}, but not if Ca^{++}, is added (47). Similarly, the viscosity effect of the polyphosphates is not produced when Mg^{++} is replaced by Ca^{++} (79). Experiments by various authors indicate that, at rather high concentrations (0.01 M), Ca^{++} seems to compete with Mg^{++}, thus decreasing the velocity of shortening of threads and of glycerol-extracted fibres and diminishing the extent of the superprecipitation of a gel (16, 33, 96). In some of these experiments, the ratio Ca^{++}/Mg^{++} must have been very high, for no Mg^{++} was added to the amount present in the actomyosin itself. In spite of this low concentration of Mg^{++}, Ca^{++} did not affect superprecipitation in concentrations below 0.01 M (47). In the experiments made by Edmann (28), however, the Ca^{++}/Mg^{++} ratio at which the velocity of shortening of extracted fibres was decreased was 1/50. This rather weak competitive action of Ca^{++} is in accordance with results obtained with actomyosin solutions. At high ionic strengths Mg^{++} acts as a strong inhibitor for the dephosphorylation of ATP, whereas Ca^{++} has an activating effect (5). With a Ca^{++}/Mg^{++} ratio of 10/1, however, a 100 to 75 per cent inhibition was found; it was reduced to 50 per cent only at a Ca^{++}/Mg^{++} ratio of 400/1 (81, 106).

As mentioned previously, very low concentrations of Ca^{++} (0.0002 M) abolish the effect of relaxing factors, such as the Marsh-Bendall factor (11), Bendall's myokinase extract (13), the factors responsible for the Bozler effect with high concentrations of ATP (24), as well as with creatinephosphate and low concentrations of ATP (23), the creatinephosphokinase system (41), and pyrophosphate in low concentrations combined with ATP (12). In this case, Ca^{++} completely abolishes the action of Mg^{++} necessary for "factor" activity though the concentrations of Ca^{++} are one tenth, or even less, of those of the Mg^{++} present. This suggests an explanation of the effect of Ca^{++} on living muscle fibres when Ca^{++} is injected into the fibre. Heilbrunn (55) found that a living muscle fibre contracted irreversibly to about one half of its original length when injected with Ringer solution containing about 0.002 M Ca^{++}. The injection of Ringer solution in which the Ca^{++} was replaced by Mg^{++}, or contained neither Ca^{++} nor Mg^{++}, did not lead to shortening of the fibre.

c) *Other divalent ions.* According to Bowen (17), Co^{++} and Mn^{++}, in the presence of 0.1 M KCl, accelerate the velocity of shrinking of actomyosin threads to the same extent as does Mg^{++}. The activation by Co^{++} decreases when the optimal concentration of 0.001 M is exceeded. On the other hand, the action of Sr^{++} and Ba^{++} is related to that of Ca^{++}. Sr^{++} and Ba^{++}, in the absence of added Mg^{++}, reduce the velocity of shrinking of the threads but to a slightly lesser extent than Ca^{++}. Since Ba^{++} forms an insoluble salt with ATP, its effect might be explained as being due to the removal of ATP from the solution.

Mn^{++} activates the dephosphorylation of ATP, although to a lesser extent than does Ca^{++} or Mg^{++} (17). The rate of splitting of ATP in 0.1 M KCl is not increased by the addition of Co^{++} and is slightly inhibited by Sr^{++}. A similarity between the action of Ca^{++} and Ba^{++} was observed by Bozler (23) who reported

an inhibition of the relaxation of the extracted fibres by traces of Ba^{++} left in the ATP preparation.

It has not yet been investigated, whether or not Mn^{++} and Co^{++} may replace Mg^{++} as a cofactor of contraction, *i.e.*, whether or not they will activate the contraction of the models after the removal of the Mg^{++} with EDTA. Possibly Mn^{++} and Co^{++} produce an additional activation only, comparable to the action of the alkali ions. The optimal concentrations, however, are lower than those of the alkali ions, even if expressed in terms of ionic strength.

d) Ethylenediaminetetraacetic acid (EDTA), hexametaphosphate, oxalate and fluoride. The inhibiting effect of these metal complexing agents decreases with decrease in their ability to bind Mg^{++}. Hexametaphosphate and EDTA strongly inhibit the contraction of muscle and cell models and the splitting of ATP by muscle models (20, 47, 64, 89). (Whether or not the splitting of ATP by cell models is inhibited remains to be investigated.) Inhibition of the contraction of the models, and of the splitting of ATP, can be reversed if Mg^{++} is restored (47, 90), while the ATP splitting returns on addition of Ca^{++} (47). Even in the presence of phosphate, NaF inhibits muscle models only in very high concentrations and the inhibition is incomplete. The inhibition can be reversed by the addition of Mg^{++} (49).

Consistent with the observations on Ca^{++} effects, oxalate does not affect the contraction of an actomyosin gel (49) or the splitting of ATP in the absence of the relaxing factors. However, it increases the activity of relaxing factors, *i.e.*, it augments relaxation (51).

3. Salts of alkali metals. The alkali ions activate the contraction of the models and the dephosphorylation of ATP (5, 16, 85, 102, 103). However, they cannot replace Mg^{++} as the cofactor for contraction or dephosphorylation of ATP, *i.e.*, models pretreated with EDTA do not regain activity by the addition of alkali ions (89). At higher concentrations, the alkali ions increasingly inhibit contraction as well as the Mg^{++} activated ATPase activity and, to a lesser degree, the Ca^{++}-activated ATPase (5, 47, 85, 102, 103). The effect of all alkali ions on the models is rather similar. The contraction of the models remains unchanged in all respects if Na^{+}, instead of K^{+}, is added (35, 87, 117), even when the fibres have been thoroughly washed with NaCl solutions; this procedure removes practically all of the K^{+} (45). However, in contrast to the models, the splitting of ATP by the actomyosin sol in the presence of very high concentrations of alkali ion (0.05 M) is affected differently by K^{+} and Na^{+}. At 0.6 M NaCl, in contrast to 0.6 M KCl, ATP is split at a very low rate (5, 19).

The values given in the literature for the minimum salt concentrations at which maximal activity occurs vary from 0.03 M to 0.1 M, depending on the models investigated and the concentrations of ATP and Mg^{++} used (16, 85, 96, 102, 103, 104). Similarly, the values at which the salt concentrations commence to have an inhibiting effect depend on the concentrations of Mg^{++} and ATP. In this regard, the particular phenomenon investigated must be taken into account. Maximal tension and extent of shortening, on the one hand, and, rate of shortening and splitting of ATP on the other, might be expected to show a dif-

ferent sensitivity towards conditions which are not optimal (see footnote 3). Thus, the values for superoptimal concentrations of alkali salts vary from 0.15 M for actomyosin gels at 0.001 M ATP and Mg^{++} to 0.5 M for the extent of shortening of fibre models (47, 85, 96, 102, 103, 104). Further increase of the salt concentration brings the actomyosin into solution (52, 102, 103).

The cell models react in a similar manner. Too high a salt concentration prevents the contraction of the cells (64, 65).

With respect to actomyosin models, Szent Györgyi and his school observed that, with decreasing atomic weight of the alkali ions, the concentrations of salts for optimal activity are lowered, whereas the optimal concentrations of anions increase with decreasing atomic weight (99, 101).

4. Compounds reacting with sulfhydryl and amino groups. a) Sulfhydryl reagents. A number of reagents which are known to react readily with SH-groups have been found to decrease, or to abolish, both the enzymatic activity and the contractility of actomyosin. Their effect could be partly or completely reversed by cysteine or *BAL* (1,2-dithiopropanol). This fact forms the basis for the assumption that SH-groups are among the reacting groups of the protein. After the discovery of the action of some thiol-binding reagents on the ATPase activity of myosin (14, 39, 43), Bailey and Perry undertook the first systematic studies on actomyosin solutions. They compared the inhibition of the enzymatic activity with the disappearance of SH-groups as indicated by iodine titration (4). Since enzymatic activity in the gel, as well as in the sol, appears to depend on SH-groups and, since analogous studies on the gel have not been performed, the results on the ATPase activity of the sol will be outlined here. Hydrogen peroxide, or iodosobenzoate, and p-chloromercuribenzoate react with some of the SH-groups very readily, even in low concentrations, and, at the same time, strongly inhibit the ATPase. The residual activity appears to be connected with the SH-groups which are less easily attacked. This activity diminishes only gradually with increasing concentration of the inhibitor. Iodine and some alkylating reagents react uniformly and slowly with all SH-groups. Thus, the activity decreases in inverse proportion to the total number of the remaining SH-groups.

Godeaux (39), and later Kuschinsky (72), Buchthal (25), Korey (71), Dickens and Glock (27) and Hasselbach (49) studied the inhibition of contraction and superprecipitation of actomyosin gels by SH-reagents (Table 1). Heavy metals completely inhibit contraction and splitting of ATP in very low concentration. With the exception of those caused by Zn^{++}, the changes produced by the free ions are irreversible. In contrast, the effect of the organic compounds of the heavy metals can be reversed by the addition of cysteine, glutathione and BAL. As was found with other native proteins, some mild oxidizing reagents, such as potassium ferricyanide, are quite ineffective, while H_2O_2 in the presence of traces of iron, porphyrexide, iodine, iodosobenzoate and potassium permanganate inhibit as strongly as the heavy metals. Dickens and Glock (27) proved that the oxidation by H_2O_2 takes place only when catalyzed by traces of iron which probably were present in the myosin preparations of the earlier investigators.

The SH-groups, which have to be free for the interaction between ATP and

actomyosin, do not seem to be easily alkylated. Iodoacetate does not inhibit at all, even in very high concentrations, while iodoacetamide and dichloren (bis-β-chloroethyl methylamine) affect contraction only in relatively high concentrations and rather slowly. Maleate, which forms an addition compound with

TABLE 1

Effect of various sulfhydryl reagents on the activity of models

SH-reagents	Concentration	Splitting of ATP		Contraction				Reference
		Model system	Inhib.	Model system	Phenom. observed*	Inhibition % **	Inhibition Reversibility	
	μM/cc.		%					
1. Ions and complexes of heavy metals								
Ag^{+}	0.01	myosin sol	100					32
	1.0			fibre	T	+	0	49
Fe^{+++}	6.0	myosin sol	35					2
	0.5			fibre	T	+	0	49
Hg^{++}	0.1			thread	Shr	100		39
	0.1			fibre	T	+	0	49
	0.1			fibre	S	+	0	71
Cu^{++}	0.3			fibre	T	+	0	49
	6.0	myosin sol	100					2
Cu-glycine	0.5	actomyosin gel	80	actomyosin gel	Spp	100	+	108
	1.0			fibre	T	+	+	49
Salyrgan	0.01–0.3	myofibrils	0–100	myofibrils	S	0–100	+	89
	0.01			myofibrils	VS	98	+	89
	0.4			cells	S	+	+	64
	0.4			actomyosin gel	Spp	100	+	73
	0.1			fibre	T	+	+	88
Zn^{++}	4.0			fibre	T	+	+	49
Oxarsan†	5.0	myofibrils	80	myofibrils	S	100	+	89
	5.0			cells	S	+	+	64
	2.5			actomyosin gel	Spp	70	+	108
p-Chloromercuribenzoate	0.01–0.2	myosin sol	0–100					4

TABLE 1—*Continued*

SH-reagents	Concentration	Splitting of ATP		Contraction				Reference
		Model system	Inhib.	Model system	Phenom. observed*	Inhibition		
						%**	Reversibility	
	μM/cc.		%					
2. Oxidizing reagents								
o-Iodosobenzoate	1.0	myosin sol	90	fibre	S	+	+	4, 71
$H_2O_2 + 10^{-4}$ M Fe^{+++}	10.0	myosin sol	0	fibre	T	0		27, 49
	100.0			fibre	T	+	0	49
$KMnO_4$	0.1			fibre	T	+	0	49
I_2	0.035	myosin sol	80					4
	5.0			fibre	T	+	0	49
K_3FeCN_6	10.0			fibre	T	0		49
	10.0			thread	Shr	0		25
Porphyrexide	1.0			thread	Shr	+		25
3. Alkylating reagents								
Iodoacetamide	4.0	myosin sol	90					4
	100			thread	Shr	+		39
Dichloren	20.0			fibre	T	+	+	49
Iodoacetate	50.0			fibre	T, S	0		49, 71
	300.0	myosin sol	0					82
Bromoacetate	30.0			thread	Shr	0		39

* T = tension, S = extent of shortening, VS = velocity of shortening, Spp = superprecipitation, Shr = shrinking of unstructured threads.

** + if only inhibition is stated without quantitative data.

† m-amino-p-hydroxyphenyl arsinous oxide.

SH-groups, does not change the activity of actomyosin. Glycine has no effect on the contractility of the models, although Greenstein and Edsall (42) observed that glycine produced in myosin a complete disappearance of the SH-groups titratable with porphyrindin.

The enzymatic activity of the models, where measured, was likewise depressed. Sometimes, however, some residual ATPase activity remained although the contractility was completely abolished (89, 108). This might be due to one of two reasons. First, the rate of energy supply may be too low to maintain a contrac-

tion; second, the energy may no longer be utilizable for the contraction. In some cases the rate of energy liberation due to the splitting of ATP, which remains after an appropriate partial poisoning with SH reagents is higher than that of the unpoisoned model at 0°C. (89). However, in contrast to the poisoned model, the unpoisoned model contracts at 0°C. This suggests that the contraction is inhibited because the energy released by the dephosphorylation of ATP cannot be utilized for contraction.

There is not enough evidence to state whether or not free SH groups are essential for all of the interactions between ATP and actomyosin, *e.g.*, the binding of ATP, or are essential only for some final steps leading to the dephosphorylation of ATP and the contraction of actomyosin. The first relaxation of models was produced in the presence of salyrgan and ATP (88, 122). A model contracted isometrically with ATP and afterwards washed with saline will not relax if salyrgan is added. If, however, salyrgan is present together with ATP, relaxation will occur immediately. This plasticizing effect of ATP suggests that ATP can still react in some way with actomyosin, although splitting of ATP and contraction are abolished completely. Salyrgan is the only heavy metal compound studied which produces relaxation in combination with ATP. With many other metal compounds, for instance copper glycine, the inhibited model is in a state of rigor (49).

b) *Amino group reagents.* Benzaldehyde abolishes contraction completely and irreversibly. It has a plasticizing effect even in the absence of ATP (72, 88).

IV. EFFECT OF SUBSTANCES WITH UNKNOWN MECHANISM OF ACTION

1. Inhibitory reagents. a) *Urea.* Bozler (21) observed that urea decreases the tension developed by glycerol-extracted fibre bundles. In the presence of urea, the degree of shortening of myofibrils is reduced to 40 per cent, instead of to 80 per cent, of the original length (89). Likewise, the cell models shorten to a lesser extent (64). With the cell models Hoffmann-Berling (64) reported that the inhibitory action is enhanced by higher concentrations of ATP, an effect which was not obtained with muscle models (89).

b) *Substances containing sulfonic groups.* Among the substances containing sulfonic groups, fuadin (sodium antimono-catecho-disulfonate), germanin (Bayer 205), trypan blue (sodium ditolyldisazobis-8-amino-1-naphthol-3,6-disulfonate), trypan red, and liquoid Roche were studied (64, 89, 90). They reduce the extent of shortening of extracted myofibrils and of glycerol-extracted cells. The effective concentrations of fuadin are higher (0.01 M) than those of germanin (0.005 M). The inhibition caused by fuadin is completely reversible, whereas the effect of germanin is only partially reversible by washing. The action of fuadin was ascribed to the antimony by Kuschinsky and Turba (108), who first observed its effect on actomyosin. Cysteine has no influence on the inhibition (89) in contrast to BAL (64, 108) which precipitates the compound.

2. Activating compounds. Cardiac glycosides. The glycosides ouabain, lanatoside C, and digoxin are reported to have a slight effect on both the speed and the extent of the contraction of glycerol-extracted fibre bundles and myosin threads.

Bowen (18), and also Edmann (28), using a statistical evaluation of a large number of experiments, reported a slight acceleration of the contraction. Bowen found that the half-time of the contraction of actomyosin threads decreased from 54 seconds to 47 seconds (1 μg. digoxin/cc.). Edmann noted no effect of ouabain in the absence of Ca^{++} and a slight increase in the extent and the speed of the contraction of glycerol-extracted fibre bundles in the presence of 0.001 M Ca^{++}. According to Edmann, the optimal concentration of ouabain is 10^{-6} M and there is a decreasing effect with higher concentrations. Mallow and Robb (75) reported an increase in the degree of shortening of actomyosin threads with "crystalline cardiac glycoside" 1:2,000,000. These experiments are difficult to evaluate, because the threads formed spirals while contracting. The maximal extent of shortening must therefore have been difficult to measure and it is not stated how this was done. Contrary to these observations, no influence on the contraction of glycerol-extracted fibres was found with digoxin (0.2 mgm/ml) (71), strophanthin (2 mgm/ml) (49), and ouabain in concentrations above 10^{-5} M (28).

3. Compounds acting on muscle models containing Marsh-Bendall factor. Caffeine and quinine. Caffeine and quinine have no influence on the contraction of the muscle models in the absence of the Marsh-Bendall factor (49, 71, 103). In its presence, however, they have an effect similar to that of Ca^{++}. They completely abolish the activity of the relaxing factor, thus preventing relaxation and causing contraction (50, 51).

4. Drugs without any influence on the contraction of muscle or cell models. A number of drugs known to influence the contraction of living skeletal or smooth muscle, *e.g.*, epinephrine, acetylcholine, nicotine, veratrine, histamine, papaverine, were found not to exert an effect on tension or shortening of fibre models (35, 49, 71, 115). Colchicine, although it affects living cells, did not inhibit the ATP contraction of cell models (64). The action of these drugs has not been investigated in the presence of relaxing factors. The evidence suggests that they do not directly influence the reaction between actomyosin and ATP.

SUMMARY

The contraction of both muscle and cell models is the result of the reaction of the contractile protein with ATP. The contraction may be studied by observation of the change in shape in the case of the cell models, of the superprecipitation of actomyosin gels, and of the unloaded shortening of actomyosin threads of glycerol-extracted fibres. It may also be studied by determining the tension produced by a model under isometric conditions, or by measuring the work done by a model. The extent of the contraction or the final tension, as well as the rate of shortening, may be investigated. It must be borne in mind that conditions affecting contraction have been shown to influence these reactions in a different way. Decrease of the Mg^{++} concentration, for instance, markedly affects the velocity of contraction of a muscle model, but has much less effect on the extent.

The contraction depends in a complex way on the active concentrations of the

factors and cofactors of the system, such as actomyosin, ATP, Mg^{++}, ionic strength and pH. Any change influencing the concentration of one of them will indirectly affect the process (*e.g.*, EDTA, hexametaphosphate, fluoride, etc.). Interpretation of the marked difference in the effects of various concentrations of ATP is complicated by the importance of the diameter of the models. A model containing a core which is free of ATP will develop a lower tension, or shorten more slowly, than will a thinner model. In a solution with a high concentration of ATP, the outer part of the model might react to a superoptimal concentration of ATP, while the center has not been reached by ATP.

The complexity of the reaction is also demonstrated in the behaviour of the enzymatic activity. With increasing ionic strength, the enzyme changes from a Mg^{++}-activated to a Mg^{++}-inhibited form. When, at low ionic strength, the actomyosin enzyme is present in its Mg^{++}-activated form, contraction is inhibited by Ca^{++} if its concentration increases far above that of Mg^{++}.

The presence of the relaxing factors drastically changes the reaction of the system in that concentrations of ATP and Mg^{++} which otherwise produce contraction bring about relaxation. While the addition of an ion, such as Ca^{++} in low concentrations, has no influence on the contraction of pure actomyosin, calcium ion in the presence of a relaxing factor initiates contraction.

As previously stated, some substances act by altering the effective concentration of the components of the system, others attack the sulfhydryl groups, or the amino groups, thereby modifying the contraction. In addition, several other substances, *e.g.*, urea, compounds containing sulfonic groups, have been found to influence contraction of the muscles reversibly by, as yet, unknown mechanisms.

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