MYOSIN AND ADENOSINETRIPHOSPHATE IN RELATION TO MUSCLE CONTRACTION

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The conception of energy provision by the splitting off of the terminal phosphate group of ATP, under the influence of myosin or actomyosin acting as ATPase, is central in current hypotheses of muscle contraction. Indeed, in many aspects of metabolism we find evidence that ATP serves as a readily expended store of energy and that much of the free energy of oxidation and glycolysis goes to its resynthesis. In these circumstances, it is strange to reflect that we are still without accurate knowledge of the amount of free energy available in this reaction; we do know, however, that it is surprisingly small, only of the order of 12000 g cals per g/mol H_3PO_4 set free. Still more surprising is the small difference in free energy content (only about 6000–8000 g cals) which separates the "energy-rich" phosphate bonds from the "energy-poor" phosphate bonds. It is probably because of its ability to deal in these small stages of energy transfer that the living cell achieves its high efficiency. Thus even normal aerobic contraction is about 20% efficient when tension production or work performance is compared with heat production; and anaerobic contraction about 40% efficient. The anaerobic recovery phase (when creatinephosphate formation is going on at the expense of carbohydrate breakdown to lactic acid) is over 90% efficient: there is little heat production during this period and the formation of the energy-rich phosphate bonds goes on with scarcely any waste in the form of heat. We shall return to this point later.

The fact that no breakdown of ATP has been demonstrated in normal contraction, but only becomes observable in fargoing fatigue, has recently been emphasized by A. V. HILL[1]. By the use of the new micro-methods, for example those of KALCKAR[2], it should now be possible to estimate ADP in amounts of the order to be expected during a single twitch or a very short series of twitches. Although rephosphorylation by means of creatine phosphate probably follows with great rapidity, by using slow-moving muscle at low temperature it might thus be possible to detect a period of ATP breakdown unobscured or only partly obscured by resynthesis.

ATPASE ACTIVITY *in vivo* AND *in vitro*

The close connection of ATP breakdown with energy provision for contraction once conceded, two very important questions arise — the exact conditions of the ATPase activity and its timing.

That myosin can act as ATPase is wellknown[3] but, as BAILEY has shown[4], the optimal conditions for the activity of myosin prepared in the classical manner and containing little actomyosin, are not those to be expected within the muscle fibre. The

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activity is very low around p_H 7.4, the activity of fresh preparations increasing progressively up to and beyond p_H 10; Ca^{++} is an essential activator and Mg^{++} exercises a strong antagonism to Ca^{++}[5]. These facts have led MOMMAERTS AND SERAIDARIAN[6], to repudiate the possibility that ATP can break down in the fibre at more than a small fraction of the rate required to produce the increase in free phosphate observed on contraction. But here some recent experiments of KEILLEY AND MEYERHOF[7] seem likely to throw important light on a dark place. In a study of the ATPase activity of various protein fractions from muscle, they found with myosin alone the high p_H optimum and the Ca^{++}-Mg^{++} antagonism already mentioned; but with actomyosin (made from "crystalline myosin" and purified actin) they observed in presence of Ca^{++} an optimum activity around p_H 7.7, almost unaltered by addition of Mg^{++}. SZENT-GYÖRGYI[8] had already remarked on Mg^{++} activation of the ATPase activity of "impure natural actomyosin" but this effect may have been due to presence of myokinase. MOMMAERTS AND SERAIDARIAN[6] report experiments on ATPase activity of actomyosin at p_H 7.0 and p_H 9.0 where Mg^{++} showed its antagonistic effect to Ca^{++}. It certainly seems that further enzymic examination of actin, myosin and their combinations might lead to illuminating results.

KEILLEY AND MEYERHOF[7] describe also the preparation from muscle of a second Mg^{++}-activated ATPase, p_H optimum 6.8, containing no myosin or actin, but possibly associated with mitochondrial particles; this may correspond to the ATPase found in the mitochondria of other tissues (SCHNEIDER[9]) but not yet so thoroughly investigated.

It is clear from this study that it would be a difficult matter to specify at present the optimal conditions for the muscle ATPase activity. Further it has to be remembered that there is considerable evidence (to be discussed later) for localization of materials in the muscle fibre. This applies to the adenylic compounds and to inorganic salts, so that we cannot assume that the ionic concentrations where the enzyme is acting *in vivo* are the same as the overall ionic concentrations. Nor have we data from which to gauge the extent of p_H variation within the fibre.

THE TIMING OF ATPASE ACTIVITY *in vivo* AND THE EFFECT OF ATP ON MYOSIN

We come now to the timing of the ATPase activity: does it occur simultaneously with contraction or with relaxation? With this is bound up the whole question of the details of interaction between myosin and ATP. Does ATP enter into combination with myosin as a result of the stimulus or is it always in some kind of combination with some part of the myosin chain? Does the ATP in combining with the myosin act as a trigger to set off the energy liberation and the shortening of the myosin? Do tension development and work performance depend on simultaneous ATP breakdown? Or does the energy liberated in contraction come in the first place from energy stored in the myosin chains, the energy from ATP dephosphorylation being used during relaxation to reconstitute the chains in their initial state?

None of these questions can be answered with assurance. We shall consider briefly the results obtained from experiments *in vitro* on the effect of ATP on myosin and actomyosin since it is from further pursuit of such analytical procedures that we can best hope to get a clue to the intimate mechanism of contraction. But at the present time perhaps the best indication of an answer to any of these questions comes, not from any results *in vitro* but from the fact that, in the living muscle, relaxation gives the impression

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of being an active process. For example, during onset of fatigue, it is the relaxation phase which becomes slowed rather than the contraction phase. This would suggest an answer in the affirmative to the last question.

THE EFFECT OF ATP ON MYOSIN SOLS

The experiments, during 1941 and 1942 of J. NEEDHAM and his collaborators in Cambridge[10] and of SZENT-GYÖRGYI and his collaborators in Szeged[11] showed the highly specific reversible effect of ATP in diminishing the double refraction of flow and the viscosity of solutions of myosin (prepared in the classical way) in 0.5 M KCl. The decrease in the length to breadth ratio of the micelles thus indicated was traced by the SZENT-GYÖRGYI school to the splitting of actomyosin; and the isolation of the new muscle protein, actin, by STRAUB[12] followed.

In a recent publication, JORDAN AND OSTER[13] have described experiments on the light-scattering properties of solutions of classical myosin in 0.5 M KCl before and after addition of ATP; they interpret their data on the change in ratio of forward to backward scattering as showing an increase in coiling of the protein particles, these being present, before ATP addition, in the form of slightly coiled rods.

The validity of this interpretation depends upon the presence of the actomyosin in the solution in the form of discrete rods and not in the form of the branching network to be seen in electron micrographs. It is very possible that the dilute solution used did contain rod-shaped particles especially as it had been subjected to ultra centrifugation, which might be expected to carry down the network.

An increased coiling of such actomyosin particles (or of myosin particles formed from them) under the influence of ATP would obviously be of importance in considerations of muscle contraction and further work along these lines, including observations on pure myosin (myosin A), will be of much interest.

It is a matter too, for future experiment to decide whether evidence for the increased coiling of the rods (after they are set free from the network) can be obtained from electron micrographs. So far attention has been concentrated on the behaviour of the network with ATP and the appearance of the resulting débris has not been closely studied.

Another step forward in our knowledge of the interaction of myosin, actin and ATP was gained by the observations of BAILEY AND PERRY[14] on the effect of –SH reagents. They showed a close correlation between the effect of reagents which oxidize or combine with –SH groups in inhibiting ATPase activity of myosin on the one hand and its power to combine with actin on the other. Thus certain –SH groups of myosin are necessary for its combination with ATP (and this is in line with much other information about enzymes concerned with ATP). These same –SH groups are necessary for combination of myosin with actin, and if ATP is added to actomyosin it displaces actin from these groups and itself combines. These results are important, not only in throwing light on the mechanism of the dissociation effect. The earlier experiments of the NEEDHAM and SZENT-GYÖRGYI groups had indicated an interaction between ATP and the protein responsible for the double refraction of flow and the high viscosity; that is to say, they made it unlikely that ATPase activity of the myosin preparations was to be put down to presence of small amounts of some other protein. This line of argument is strongly re-inforced by the work of BAILEY AND PERRY which forms the best evidence so far

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for the ATPase activity of myosin itself. The knowledge gained from the study of interaction between ATP and myosin sols must clearly play a useful part in our progress towards understanding of muscle contraction. But it does not seem that any deductions having a direct bearing on the question we have raised can be drawn from it at the moment. Certainly a deduction recently made from the results of DAINTY *et al.*[15] by MORALES[16] that "the catalytic activity of ATPase, that is of acto-myosin, rises exponentially with disorientation of the protein" is not justified.

THE EFFECT OF ATP ON MYOSIN THREADS

The similarity in rod and intrinsic birefringence and in the X-ray diagram between artifical myosin threads and muscle fibres led to hope that important progress might be made by study of the effect of ATP upon such threads; especially since it was found that they still retain ATPase activity and could withstand a certain amount of tension (ENGELHARDT[17]) without breaking.

ENGELHARDT used threads made from classical myosin and containing about 2% protein. Subjected to loads of about 200 mg such threads show a reversible extension. If the threads are tested, immersed not in KCl solution but in 0.005 M ATP, this extensibility is increased by 50–100%.

This effect of rise in extensibility with loaded threads is in contrast to the striking shortening effect obtained by SZENT-GYÖRGYI[11] with unloaded actomyosin threads (myosin B), suspended in dilute (0.05 M) KCl. Addition of ATP (0.002 M) led to isodimensional contraction, with shortening up to 66%. This shrinkage of the actomyosin thread is accompanied by great loss of water, the percentage falling from about 97 to 50.

The observations of BUCHTHAL *et al.*[18] form a link between these two sets of observations. Using actomyosin threads (which had been dried to a N content of 16.15% and then allowed to imbibe water for 30 minutes from 0.9% NaCl solution) they found that addition of 0.002 M ATP caused a 30% shortening of the unloaded thread; while with a load of 110 mg there was an increase in length of 30%. Even so small a load as 5 mg caused a slight lengthening.

PERRY *et al.*[19] have contributed an instructive electron microscope and X-ray study of the synaeretic effect of ATP on actomyosin gel in 0.05% KCl. The photographs of the control gel show a dense tangled network. After ATP addition, the network has opened out; it would appear that small linear fibres are first formed (as might be expected on a splitting to actin and myosin) and that these aggregate side by side to form denser fibres. The X-ray diagrams from the same material show no fundamental difference between actomyosin before and after synaeresis. These observations were taken to indicate that there is no increased intramolecular folding with intramolecular synaeresis, but rather that the water loss is intermolecular accompanied by lateral aggregation.

When all these facts are considered together it seems that the discrepancy originally felt between the results of ENGELHARDT and of SZENT-GYÖRGYI disappears. The effect of the ATP in both cases is to cause breakdown of the actomyosin network followed by aggregation of the particles and squeezing out of water. When the thread is loaded, the fall in elasticity consequent upon the disappearance of the network is the obvious aspect; when the thread is unloaded, this aspect is not noticeable but the shortening due to synaeresis can manifest itself.

BUCHTHAL *et al.*[18] have reported that treatment of fresh actomyosin threads (3%

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protein) with the sulphydryl reagents iodoacetate and porphyrexid causes decreased shortening when ATP is subsequently added. The interpretation of these results is not immediately obvious for, when sulphydryl reagents are added to actomyosin sols in 0.5 M KCl, there is a decrease in viscosity as would be expected if the reagent, like ATP, broke the link between the actin and the myosin. The effect is slower than with ATP itself, probably because the reagents react also with other –SH groups (for example, in the actin) while the ATP reacts specificially with the connecting groups. In the case of the threads, there seems no reason why –SH reagents should inhibit the splitting of the myosin from the actin; possibly the attachment of ATP to the myosin, as well as the presence of free –SH groups, is involved in the further changes in state of aggregation and it is these which become impossible.

It seems doubtful whether these phenomena of synaeresis are connected with the mechanism of contraction. If the removal of water is intermolecular, this would lead *in vivo* to a narrowing rather than a shortening of the fibres, since both myosin and actin are known to be arranged with their long axes parallel to the fibre axis. PERRY *et al.*[19] have remarked on this and also pointed out that though the loss of water associated with volume contraction is very rapid, the reverse process (which might be analogous to relaxation) is slow and there is little information as to its degree.

THE LOCALIZATION OF SUBSTANCES IN THE STRIATED FIBRE

The anisotropic (A) band seems to have had, from the early days of work on muscle fibres, a particular interest for observers. In spite of the many variations and discrepancies of description of the histological appearance of contracted muscle (depending on the different sources of the muscle; differences in preparation, whether fresh or fixed and stained; differences in optical set-up; and differences in degree of contraction) there has been a widespread if by no means unanimous opinion that it is the A-band which becomes shorter in appearance on contraction of the muscle while the I-band may show little change or even become longer.

This conclusion was probably based partly on the formation of contraction bands (see below) in strong contraction; a condition where the position of the staining material has actually become reversed with respect to the A- and I-bands; but studies like those of BUCHTHAL *et al.*[20] on single living fibres do show a decrease in the A/I ratio in early contraction. The work of BUCHTHAL *et al.* was quantitative and showed, in short isometric tetani, a decrease in length of the A-band of 18%, an increase in the I-band of 28%.

The visible changes in length of the A- and I-bands have often been taken as indicating that the actual contractile process was limited to the A-bands; the I-bands, though not necessarily considered as passive, being the seat of less important changes. The conception of more recent years took the form that in the A-bands the protein micelles undergo folding while this process is much less or gives place to unfolding in the I-bands.

The idea that the protein of the A-band actually differed in kind from that of the I-band was given up as more accurate estimates of the myosin content of the muscle became available. For several years the view was then prevalent that the fibril consists of collections of myosin chains, arranged in the anisotropic bands with their long axes parallel to the fibril axis, but in the isotropic bands having much less orderly arrange-

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ment. However the recent production of electron micrographs of muscle fibres (HALL, JAKUS, AND SCHMITT[21]), showing continuous micelles passing straight through A- and I-bands, brought the realization that the lack of orderly arrangement in the I-bands (if it exists) must be at a level of dimension below the resolving power of the electron microscope. The work of DEMPSEY *et al*,[22] about the same time, demonstrated the presence in I-bands of lipoids with negative double refraction and the possibility of converting striated fibrils, by thorough extraction with fat solvent, into fibrils of uniform positive double refraction. MATOLTSY AND GERENDAS[23] also report experiments indicating the presence of a substance of negative double refraction in the I-band.

The present-day conception is therefore rather that the fibrils consist uniformly through their length of bundles of myosin (or actomyosin) chains pursuing an apparently straight course, and as far as our knowledge of these chains goes, there is no obvious reason for a localization of the contraction process in the A-bands.

We have some further knowledge indicating a high degree of localization of other substances within the fibre, and also (a matter of particular interest) in some cases suggesting a change in location during contraction. Since the possibility arises that changes in position of non-myosin material may affect the visible length of the A- and I-bands, this subject may be pursued a little further. Thus there is good evidence (CASPERSSON AND THORELL[24]) that material with selective absorption at a wave-length of 265 mμ is concentrated in the I-bands in resting muscle; in muscle after vigorous contraction there is spread of the material into the A-bands. The adenylic compounds are the most likely to be responsible for this effect; it has also been suggested that they may contribute to the negative double refraction of the I-band (BARER[25]).

Then we have the more recent work of SCOTT AND PACKER[26] (using a rapid freeze-drying method and careful avoidance of water to prevent movement of soluble salts) confirming a good deal of earlier work in finding the greater part of the ash in the A-band. There were indications that this localization applied to calcium and magnesium. Finally it has long been known that the A-bands contain material which stains deeply with basophilic dyes. This material seems to contribute to the dark colour of the A-band in fresh fibres in ordinary light, but does not seem to be concerned with the double refraction of the A-band. Histological literature abounds with detailed descriptions of the movement of this material (the A substance) during contraction. Such descriptions are usually concerned with fixed and stained material but as of more interest we may take the example of the more recent work of SPEIDEL[27] on living muscle of vertebrates and invertebrates. He describes, as the fibre shortens, first a shortening of both A and I; secondly a blurring of cross striae when the sarcomeres have shortened by about one third, as if the dark refracting material were undergoing profound redistribution or chemical change; thirdly, concentration of the dark refractive material (the contraction band) about each Z disc (crossing the centre of what was, during rest, the I-band).

It is interesting that the electron micrographs of HALL, JAKUS, AND SCHMITT[21] show material (of which we know only that it has high electron-scattering power and high affinity for phosphotungstic acid) concentrated in the resting fibril in the A-band. When fibres are stained with phosphotungstic acid and fixed in different stages of contraction, stages can be made out in the electron micrographs indicating the spreading of stainable material from the A-bands, until at about 40% shortening a state is reached with a narrow dense band in the position of the Z-membrane, the rest of the fibre, including the A-band, being uniform with comparatively faint staining. The close

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correspondence with the behaviour of the "A" substance described above is striking.

These observations may be summarized as follows:

TABLE I

I-Band	Movement during contraction	A-Band
Lipoids Adenylic compounds	——→	Salts, perhaps especially Ca and Mg
	←——	Basophilic A substance
	←——	Electron scattering substances

It seems that there must be some intimate connection between the three classes of substance mentioned in the 3rd. column; whether the same substances are actually responsible for the staining and the electron-scattering phenomena we do not know.

Besides these localizations which have been recognized, and which must have significance for contraction it seems likely that there may be much localization still unknown. In particular it is to be expected that the soluble protein fractions, myogen and globulin X, including most of the enzyme equipment of the muscle, instead of being merely dissolved in the sarcoplasm, will show pattern.

CONCLUSION

If one is to make any sort of tentative picture of the mechanism of contraction, one must, under present conditions, be allowed a bias towards one side or the other in answering the question "Is relaxation of the fibril an active process, requiring provision of free energy?" The writer would like to take the standpoint that an affirmative answer best fits the observed physiological behaviour during relaxation and that observations on the relations of heat production and on the effect of work on heat production are not at variance with this view.

One can make the basic assumptions that, in the stimulated muscle, chemical reaction becomes possible between groups situated along the protein chain; that this reaction goes on with production of free energy and that in the resting muscle there is some configurational hindrance to its taking place. Further, one can assume that the number of these groups which can react together will depend upon the length which the muscle is made to assume, being fewer at greater lengths and increasing in number as the muscle shortens. It is known that during a twitch the amount of "shortening heat" production is proportional to the degree of shortening of the muscle, while the rate of "shortening heat" production is dependent on the speed with which the muscle shortens, (A. V. HILL[28]). Thus for shortening a given distance, the "shortening heat" production is the same, whether the shortening is slow or fast. But the rate of shortening depends on the load, being slower the greater the load; thus at slower rates of shortening between two given lengths, more work is done and more energy must be produced, since the heat remains the same. If this energy production is the result of the interaction of the same groups at different rates of shortening, we must suppose that, at the slower rates, repeated interaction takes place. When speculations are made as to the timing of ATP breakdown, it is usually supposed that this is confined either to the contraction phase or to the relaxation phase (in the latter case its energy being used to restore energy-rich protein linkages). If we suppose that, when work is done, before a pair

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of groups can react together a second time, they must have been put back into their original state by means of free energy provided by reaction with ATP, we see that ATP breakdown could begin within the contraction phase, even though it were associated with restoration of the chains.

A. V. HILL[29] has shown that the relaxation phase of a twitch is free from heat, if the work done is not allowed to degenerate into heat. During this period, on the view under discussion, ATP breakdown would be continuing the process of separating the reactive groups, a process leading now to the lengthening of the fibril. Since no heat is associated with the relaxation phase, this process would seem to be 100% efficient, and the waste heat associated with the contraction phase would appear to be due to the primary reaction along the protein chains. As we have seen, the anaerobic recovery process (immediately following an anaerobic contraction) is known to go on with very little heat wastage; it is not unlikely that there is a similar efficiency in the relaxation process. A mechanism suggested for the transfer of energy (KALCKAR[30]; DAINTY *et al.*) is the transfer of phosphate from ATP to the protein chains; this still remains a possibility, (see F. BUCHTHAL *et al.*[30]).

The *Verkürzungsort* still retains its mystery but we begin perhaps to see in what direction solution lies.

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