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THE ROLE OF ADENOSINE - TRIPHOSPHATE IN MUSCLE

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Adenosine triphosphate (ATP) is the key compound from which the energy for muscular activity is derived, but besides this important rôle it is also responsible for keeping the living muscle in its normal flexible and extensible state. Considering ATP first as a contractile agent, we find that contraction is invariably associated with the splitting of ATP by the contractile protein, actomyosin, in the reaction: -

1) $A^{TP} + H_2^{O}$ $\xrightarrow{}$ $ADP + H_3^{PO}_4$ It is reasonable to conclude that it is this reaction wh

+ Mg ions

It is reasonable to conclude that it is this reaction which supplies the energy, but it is not at present known how this comes about, although it seems probable that one step must be the phosphorylation of the contractile protein.

In the resting muscle the splitting of ATP by actomyosin is controlled at a low level, so that no contraction can occur until a nerve stimulus arrives. Again, the mechanism of this control is not fully understood, although it is known that muscle contains a factor, discovered by Marsh, which inhibits splitting in model systems. This factor is associated with the finer granules of muscle, which can be sedimented at 30 to 90,000 g. In the presence of factor, model fibres, such as glycerinated fibres, do not contract on addition of ATP, but regain their characteristically high pre-rigor extensibility, that is they stretch under an applied load. Similar relaxing effects can be induced by any substance which inhibits the ATP-ase activity of the fibres. There are a large variety of such compounds of which E.D.T.A., pyrophosphate and the SH-blocking reagent, Salyrgan, are amongst the most effective. Necessary for all these relaxing effects is the presence of magnesium ions, without which ATP is ineffective as co-partner, just as it cannot bring about contraction unless such ions are present. On the other hand, addition of Ca ions overcomes the relaxing effects, and induces a rapid contraction except in the special case of SH-reagents. Ca is not, however, necessary for the contractile process itself, but merely to remove or inhibit the relaxing agent from the active sites.

These complex reactions leading either to contraction or relaxation can best be illustrated in model systems of glycerinated fibres, that is simple contractile systems from which the enzymes concerned with resynthesis of ATP etc. have been removed. First, by adding ATP and Mg ions we obtain a rapid contraction under load. Then by adding a relaxing agent, without removing the ATP, we obtain equally rapid relaxation, and then by adding a trace of Ca ions the relaxing agent is inhibited and a new contraction sets in. This cycle can be repeated by washing the fibres briefly to remove Ca ions, but not relaxing agent. On addition of ATP + Mg relaxation sets in once more, and so on. - 2 -

Just as we obtain these relaxing and contracting effects with fibres, so we can obtain similar swelling and shrinking effects with suspensions of myofibrils. Thus, we can measure the rate of sedimentation of a suspension of fibrils by the simple optical device of Marsh. Then by re-suspending the fibrils and adding ATP and Mg ions, we produce a vast shrinkage, which can be demonstrated by the much greater rate of sedimentation of the treated fibrils and by their smaller final packing volume. Thus, the shrunken fibrils which have been treated with ATP occupy about half the volume of the untreated fibrils. We can then repeat the experiment in the presence of a relaxing agent. Addition of ATP now results not in an increased, but a decreased rate of sedimentation, lower in fact than the rate for untreated fibrils, and a final packing volume 1½ to 2 times the volume of the latter. This clearly demonstrates the remarkable capacity of ATP for swelling the fibrils when their ATP-ase activity is inhibited. It is perhaps interesting to compare these swelling effects with those of other polyphosphates, such as inorganic pyrophosphate. The latter is, of course, not split by actonyosin and has no contractile effects of its own, so that addition of a relaxing agent is not necessary. This substance produces about the same swelling as ATP, but the swelling takes place over hours instead of seconds or minutes in the case of ATP. In both cases, Mg ions appear to be essential for swelling or relaxation, and cannot be replaced by Ca ions. The latter, however, overcome only the swelling effect of ATP, because they stimulate splitting, but have no inhibitory effect in the presence of pyrophosphate alone.

From what we have learnt from model systems we can begin to interpret the more complex behaviour of the living muscle, where besides the contraction/relaxation system, we have not only powerful systems for the resynthesis of ATP, but also soluble enzymes capable of splitting ATP without producing any contraction whatsoever. The latter are called the sarcoplasmic ATP-ases. In the resting muscle, we can envisage the contractile machinery lying domaant under the control of the granular relaxing factor of Marsh, and unable to split the ATP diffusing around it, because the ionic conditions are optimal for such control, that is the Mg ions are freely diffusing, whereas the Ca ions are bound to the muscle proteins. At the same time the muscle is fully relaxed and will stretch easily under light loads. It is not, however, biochemically inert, but is actually breaking down and resynthesising ATP all the time, although only very slowly. The breakdown occurs partly through the sarcoplasmic ATP-ases, which are not inhibited by Marsh factor, and partly through the demands for synthesis of various metabolites, such as glucose-l-phosphate from glucose. It is opposed by the powerful resynthetic mechanisms:-

1)	ADP + C.P	 ATP + C	Creatine phospho-kinase

ATP + AMP

2)

2 ADP

na linestan of that.

Myokinase

3) Resynthesis by oxidative phosphorylation.

4) Resynthesis in the anaerobic glycolytic cycle.

If this living system is stimulated, we may imagine the action potential, that is the reversal of charge on the outer membrane, being transferred within, possibly to release traces of Ca ions from their normally bound state, which in turn abolish control by the factor and lead to rapid splitting of ATP, of the order of 200 times the resting rate, and/subsequent contraction. As the action potential dies away, the Ca ions are recaptured, the ATP

is rapidly resynthesised, the Marsh factor regains control over splitting and relaxation ensues. At least, so we may speculate on the evidence available.

Now let us consider the dying muscle, removed from the animal and placed under anaerobic conditions. Providing the muscle has remained quiescent during removal, we shall have a situation where the ATP, C.P and glycogen levels are almost as high as they were during life, but from now on the breakdown of ATP by sarcoplasmic ATP-ases will be opposed by a gradually diminishing potential of resynthesis. Thus, although the ATP level can be maintained against these inroads for some hours after death, sooner or later it will begin to fall, and as it does so the muscle will pass into <u>rigor mortis</u>. We find on careful analysis that the ATP level falls, in fact, when (and not until) the C.P level is almost exhausted, whether or not resynthesis from glycolysis is still possible. Thus, a high initial level of glycogen is not as important in staving off the onset of rigor as a high level of C.P.

Another interesting point is that rigor occurs at different ATP levels according to the pH of the muscle. Thus, if we starve the animal, less glycogen is available and, therefore, less lactic acid is produced so that the pH does not fall below 6.5, with the result that the level of ATP at onset of rigor is higher than in the case of a well-fed animal where the pH may fall to 5.7. In the extreme case of an exhausted animal, the pH may not even fall below 7.20, and in such a case rigor sets in as soon as the ATP level falls. These effects will be illustrated. They can be explained by the observation of Briggs and Portzehl that full activity of the relaxing factor in artificial systems is obtained at lower ATP levels at low pH than at high.

Thus, from a study of the model systems, it is possible to go a long way towards explaining the behaviour of the living and dying muscle. So far, we understand the biochemical and physical changes in dying muscle far better than those in the living system, but even there the use of models has shown us the likely limits within which normal contraction and relaxation may take place. Above all, the models have shown us the overwhelming importance of ATP, which cannot be replaced by any of its homologues, such as I.T.P. or other nucleotide-triphosphates, although these can simulate some but not all of its effects. Similarly, the chief co-factor, the Mg ion, cannot be effectively replaced by other divalent cations, and particularly not by the Ca ion which has opposite effects.

When we relate these somewhat theoretical findings to the practical handling of meat, we find they have an immediate bearing in two directions, one the question of freezing and the other the improvement of the water-holding capacity of minced meat by addition of pyrophosphates. In the freezing of meat, for example, the ATP level at the time of freezing is of great importance, because if it is very high the neat will contract when it is thawed cut, whereas if rigor muscle, that is meat with a low ATP content, is frozen no such thaw-contracture occurs. Although thaw contracture rarely occurs with frozen sides of beef, where freezing is too slow to trap very much ATP, it has been observed both in the freezing of sides of lamb and of whale-meat. Its results are an unsightly, curled-up appearance of the meat and the loss of much fluid, both clearly undesirable features. So far as the water-retention of minced meat is concerned, the use of pyrophosphates is already well-established, and it is also known that their effect is greater if the meat is minced soon after removal from the animal, i.e. when the ATP-level is high.

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There are doubtless many other ways in which this work can be applied to the technology of meat, and it is hoped some of these will be mentioned at the conference.

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