Discussion.

by

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It has been shown in the previous papers that myosin can be extracted from muscle in two forms: as the relatively inactive myosin A and the very reactive myosin B. The myosin of previous investigators, prepared by EDSALL's method, corresponds to our myosin A with a small admixture of myosin B as an impurity. We have shown that myosin A is transformed into B if it stands in prolonged contact with muscle particles.

If we want to correlate our data with muscle physiology, our first question must be: what is the relation of the two substances that we called myosin A and B and what is the nature of the A→B transformation.

Experiments of F. B. STRAUB, now in progress, difinitely show that myosin B is a stoichiometric compound of myosin A and another substance. We will call this other substance „actin" and the myosin-actin complex will be called „acto-myosin". Actin, in itself, is insoluble in salt solution. It is, together with cytochromoxidase and succinodehydrogenase, part of the insoluble muscle residue.

There in thus, according to these results of F. B. STRAUB which will be presented later, but one myosin, and the substance that we called myosin B is acto-myosin.

As has been shown by MOMMAERTS and STRAUB myosin B, just as myosin A, forms a well defined complex with ATP also. Myosin can therefore exist in four different forms: 1. as free myosin (myosin A), 2. as ATP myosin, 3. as acto-myosin (myosin B), 4. as ATP-acto-myosin. If we want to discuss the influence of salts on myosin we do better if we talk about the influence of salts on these four different forms.

In table I. I am giving the table of T. ERDÖS somewhat

Table I.

Mol. KCl + 0,001 mol. $MgCl_2$	Myosin	ATP-Myosin	Acto-Myosin	ATP-Acto-Myosin
0	—	—	—	—
0,01	—	+	—	+++
0,1	—	+	—	+++
0,17	—	+	—	+++
0,18	—	+	—	+++
0,19	—	+	—	+++
0,20	—	—	—	?
0,21	—	—	—	—
0,22	—	—	—	—
0,23	—	—	—	—
0,24	—	—	—	x
0,25	—	—	—	x
0,30	—	—	—	x
0,31	—	—	—	x
0,32	x	—	—	x
0,33	x	—	—	x
0,45	x	—	—	x
0,46	x	—	—	x
0,47	x	x	—	x
0,48	x	x	—	x
0,60	x	x	—	x

completed and translated into these new terms. The signs mean the same as in ERDŐS's paper: — means inactivity *i. e.* no contraction and no dissolution, × means dissolution; ? means partial contraction, partial dissolution. The % of contraction has been marked with crosses, +++ meaning maximal, + weak contraction. There was 0,001 mol $MgCl_2$ present everywhere.

It will be seen that free myosin is fairly soluble in KCl and gives no contraction. ATP-myosin is less soluble in salt and gives a weak contraction at a low KCl concentration. Acto-myosin is insoluble in salt and gives no contraction. By forming the ATP compound this complex becomes most sensitive to salts: according to the concentration of the salt present the ATP-acto-myosin may be inactive, maximally contracted, inactive again or dissolved, and all this at a KCl concentration below 0,25 mol.

If we try to apply this experience to muscle, the first question is whether in muscle the myosin is in sufficiently intimate contact with the actin to form a complex. It has been shown by BANGA and myself that in the absence of ATP the myosin of the muscle cannot be extracted with EDSALL's fluid, which means that it is insoluble in salt solutions and behaves thus like acto-myosin. I have shown that the myosin in the frozen and extracted muscle reacts as myosin B. It can therefore be stated that myosin is in sufficiently intimate touch with the actin in muscle to form acto-myosin.

In living muscle, under normal conditions, there is always a fairly high concentration of ATP which is kept constant throughout life. The living as well as the freshly minced muscle contains thus the highly sensitive ATP-acto-myosin.

Our viscosity measurements indicate that, at the high salt concentration of EDSALL's salt solution (0,6 mol KCl), the complex dissociates into actin and ATP-myosin. This helps us to understand what happens in the 24 h. extraction. If we suspend the muscle in EDSALL's fluid, the ATP-acto-myosin dissociates into actin and ATP-myosin. The former is insoluble in salt solution or is linked to the insoluble residue and will therefore remain undissolved while the ATP-myosin goes into solution. For this reason we always obtain myosin A from fresh muscle containing ATP whether the myosin, present in muscle, was bound to the actin and was thus present as myosin B or not. On storage the ATP is split and the dissolved ATP-myosin goes over into free myosin which forms acto-myosin with actin. This complex being stable even in the presence of 0,6 mol. KCl, the myosin which is already dissolved will, by the formation of this complex, bring the actin into solution. (Possibly the connections of the actin with the residue are loosened up meanwhile by the alkaline reaction). If we start with muscle free from ATP, the myosin will be present in the form of the stable and insoluble acto-myosin which is insensitive to salts, and so will not be extracted by the salt solution.

Evidence indicates thus that myosin is present in muscle as ATP-acto-myosin. As shown by the last column of the table, this complex can exist in different states which depend on the KCl concentration: if there is no salt present the

complex will neither contract, nor dissolve, i. e. it will be inactive; on addition of very small amounts of KCl (0,01 mol) we will get (in the presence of Mg) maximal contraction. The change from 0 to 0,01 mol. concentration will be sufficient to cause the inactive myosin to contract maximally. A similar jump in the opposite direction will be observed between 0,18 and 0,20 mol, the change of 0,02 mol in the KCl concentration being sufficient to cause a maximal effect. Any of these two changes might be analogous to what happens in muscle when the wave of excitation arrives and the muscle goes over from relaxation to contraction. It is not impossible either that relaxed muscle corresponds to our dissolved myosin which is formed at 0,24 mol. KCl.

Naturally I do not mean to say that there is KCl in muscle and that the change of the KCl concentration is the cause of contraction. This would be in contradiction with primitive facts. What I mean to say is that our experiments show that slight changes in ionic concentration are capable of inducing changes in the structure of the protein and that analogous changes in the protein might occur in muscular contraction.

As contraction is brought about in our threads by a disturbance of the ionic equilibrium, so, in its turn, contraction might act on the ionic balance. It does not seem impossible that in muscle the contraction of one ATP-acto-myosin unit might cause the disturbance responsible for the contraction of the next unit and so forth. In this case the sharp distinction between the wave of excitation and contraction would be unjustified.

No attempt has been made in this series of papers to explain the inner molecular mechanism of the contraction of myosin. In our experiments the changes in the physical state of the ATP-acto-myosin-complex have been brought about by salts and are thus, to some extent, analogous to other colloidal reactions. As has been shown by BANGA and ERDŐS the splitting of ATP is not involved in the developement of the contraction. The myosin becomes fermentatively active only when contracted. Translated into terms of muscle physiology this would mean that the energy of the splitting of ATP is used for relaxation; relaxed muscle again is inactive. This causes

adequate quantities of ATP to be split and only adequate quantities of energy to be liberated, since it is the function itself (contraction) which liberates energy. Naturally I do not mean to say that contraction, as such, activates phosphorilysis. The contraction as well as the enzymic activity are consequences and expressions of the same change in the finer structure of the molecule.

I want to close with a rather subjective remark. I was always led in research by my conviction that the primitive, basic functions of living matter are brought about by ions, ions being the only powerful tools which life found in the sea water where it originated. Contraction is one of the basic primitive functions and the results reported in this volume corroborate me in my conviction.

PS. After this volume was sent to press reprints have been received from *U. D'Ancona* (Protoplasma *17*,388,1932) who emphasises the dehydration of the muscle fibril during contraction. This is in agreement with our observations on myosin.

A reprint has also been received from *Fr. Verzár* (Schweiz. Med. Wochenschr. *72*, 661,1942). He stresses the importance of K in contraction and brings the known facts about K, carbohydrates and adrenals into one ingenious theory. He also quotes *J. Needham* and collaborators (Nature 147,766,1941) who found that the double refraction of flow of myosin disappears in presence of small concentrations of KCl and ATP. Very unfortunately this paper is not accessible here at present.