Observations on muscle extracts.

by

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If the minced muscle is extracted for ten minutes with 0,6 *M* KCl and centrifuged a fluid is obtained which contains about 1—1,2% of actomyosin with a 0,5—1% actin content. If the extract is stored over night at 0° the ATP is split. If the extract is diluted now with 4 vol. of water containing 0,001 *M* $MgCl_2$, a slightly turbid fluid is obtained which contains about 0,1 *M* KCl and the actomyosin in the form of a stable suspension.* If ATP is added now, according to its concentration, a turbidity, or a clearing up will be seen. It seems to be logical to observe this before a black back-ground with side illumination. This mode of observation, however, gives in this case erroneous result for flocculation often goes hand in hand with a decrease of luminosity.

Good results are obtained if the flocculation is observed before a black background with light falling in from behind the test tube at a very small angle. To make this into a simple method the test tubes were immersed into a waterbath with glass walls. At the back wall a paper-screen with black lines was fixed (Fig. 1). The source of light was placed behind this screen. Now the light falls in from between the black lines at a very small angle and the black lines form the black background. Precipitation will cause the black strips to appear hazy and grey. Clearing up will have the opposite effect.

The ATP was always introduced with a small spoon

* The water should be added suddenly. If it is run in slowly the actomyosin flocculates.

(Fig. 1) in a volume of 0,1 ml. If the spoon is pulled once or twice through the fluid within the fractions of a second complete mixing can be obtained. Stirring was continued throughout the observation.

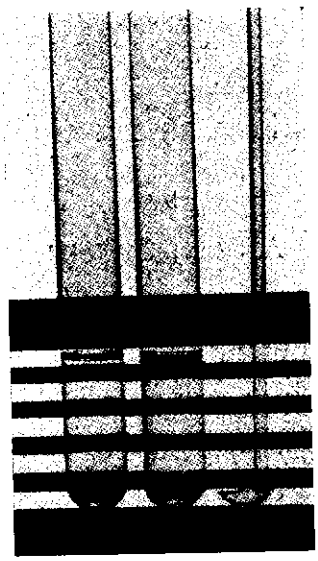

Fig. 1.

What will happen on addition of ATP depends on the first place on the quantity of the ATP added. If a very small quantity is added a precipitate will be formed at once. If more ATP is added the fluid will clear up. After the excess of ATP has been split the fluid will suddenly become turbid and precipitate will be formed.

If we plot the quantity of ATP against the time required for the formation of the precipitate we obtain the time curve of the splitting of ATP. Such an experiment is reproduced in the fig. 2. (0,1 *M* KCl). The splitting proceeds at a low ATP concentration at a fairly high rate to slow down suddenly at a somewhat higher ATP concentration. At a high ATP concentration the splitting becomes faster again. An increase of the KCl concentration from 0,1 to 0,15 and 0,2 *M* caused a flattening of the curve while the decrease of the KCl concentration from 0,1 *M* to 0,075 *M* made the curve much steeper. At 0,05 *M* KCl

the curve is still steeper. All the curves were S shaped. (On the curves of 0,15 and 0,2 *M* KCl the right half of the S falls outside the figure.)

The explanation of this change of slope is given by the experiments of I. BANGA who is showing in her paper that Mg very greatly inhibits the splitting of ATP by myosin and enhances the splitting of ATP by actomyosin. The increasing KCl concentration, as shown by F. GUBA makes the actomyosin dissociate into actin and myosin; the phosphatase action of the

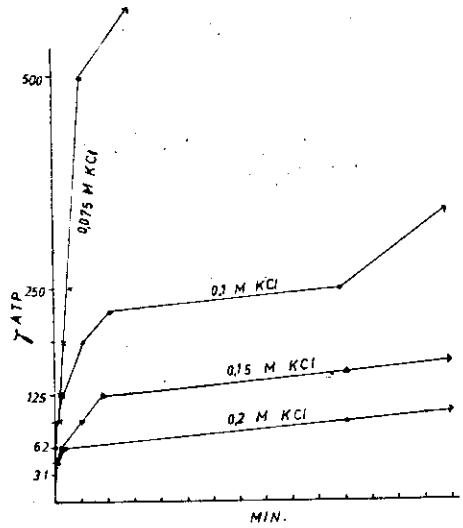

Fig. 2.

latter is then inhibited by Mg. The flattening of the curve indicates thus the dissociation of actomyosin.

This explains also the paradoxical flattening of the curve caused by the increase of ATP concentration. Evidently the excess of ATP also promotes dissociation.

A number of minor problems can be approached by the method described.

1. *ATP estimation.* If an unknown ATP solution is added to the 0,1 *M* KCl extract and its effect is compared with the effect of a known ATP solution, conclusion can be drawn on the ATP content of the unknown solution, provided it did not materially change the salt concentration.

2. *Maximum rate of splitting of ATP by myosin.* If we

measure phosphatase action by estimating the P liberated we have to take greater amounts of ATP and longer periods. Higher concentrations of ATP, as shown, inhibit the enzymic action. The described experiment allows to work with small ATP concentrations. In our experiment, working with small ATP concentrations at 0,05 *M* KCl, 100,000 g of myosin split 10 g molecules of ATP per second.

3. *Reversibility*. A small quantity of ATP causes precipitation, a bigger dissolution (0,1 *M* KCl). If we add a small quantity of ATP and, as soon as the precipitate is formed, we add a bigger dose, the precipitate will dissolve. If, however, we wait for a minute or so before adding the second ATP there will be no dissolution or the dissolution will be incomplete. This shows that very quickly unspecific cohesive links are formed in an actomyosin precipitate.