# Rigor, contracture and ATP.

by

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It has been shown in previous papers of this laboratory that the contractile substance of the muscle fibril is actomyosin, a complex composed of two proteins, actin and myosin. Actin is bound to the solid structure or rather forms the solid structure of the fibril itself. Myosin is attached to the actin in a loose, dissociable form. It has also been shown that ATP has a decisive influence on the physical state of actomyosin which, according to conditions, is contracted, relaxed or dissolved by ATP.

The object of this work was to see, whether in muscle ATP has any influence on the physical state of the actomyosin, especially, whether the relaxed state of the muscle is dependent on its presence.

Muscle contains the rather high concentration of 300—350 mg % of ATP. CASPERRSSON and THORRELL[1] have shown that a considerable part of the ATP is bound in the *I* band. One can thus expect no close parallelism between ATP concentration and the state of relaxation because the ATP is not evenly distributed. A really close parallel can be expected in rigor mortis only, where there is a post mortal disorganisation and sufficient time for the ATP to be distributed evenly within the muscle fiber. In other cases, the more the muscle is damaged and the more time given for even distribution of ATP the closer we may expect the parallelism to be. Apart from rigor mortis I also studied other forms of contracture, such as monoiodoacetic acid, caffeine, chloroform-contracture and the contracture of electrically stimulated muscle.

It has been found by several investigators (2, 3, 4) that the solubility of muscle proteins decreases during activity and in different forms of contracture. In the case of monoiodoacetic

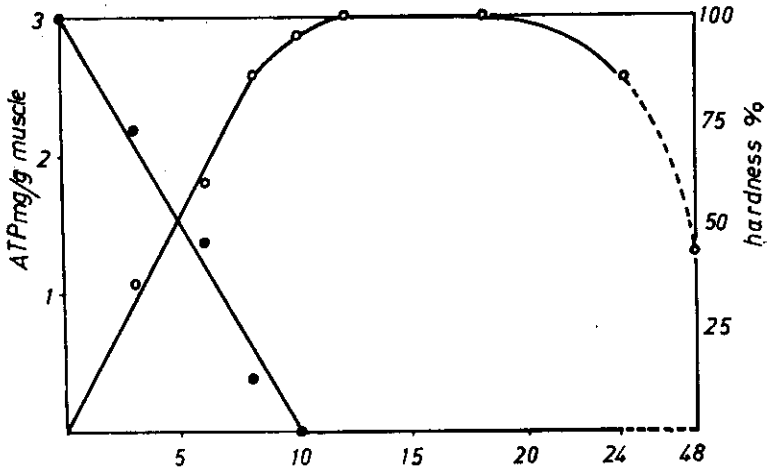


Fig. 1. a. ATP content during development of rigor mortis, in rabbit muscle.
● Hardness %/0%: fresh muscle. 100%: rigor mortis.
○ ATP mg/muscle

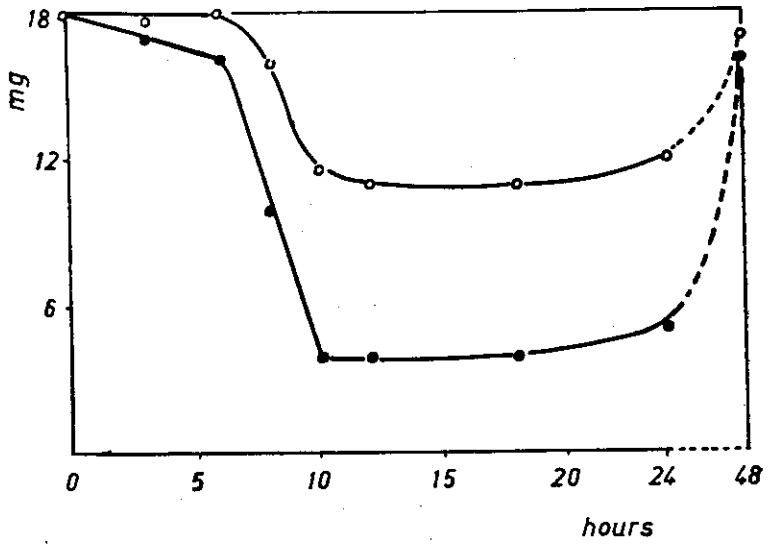


Fig. 1. b. Solubility of myosin during the development of rigor mortis. (Same experiment as in Fig. 1. a.)
● Myosin mg/ml. extracted with 3 vols. of. 0.6 *M* KCl.
○ Myosin mg/ml. extracted with 3 vols. of 0.6 *M* KCl. in presence of 100 mg% ATP.

acid, MIRSKY[5] found that the solubility of myosin is decreased. For this reason I also undertook to measure, parallel with the rigor and the ATP concentration, the solubility of myosin. My results were the following:

*Rigor mortis:* There is a close parallelism between hardness of the muscle and the ATP concentration. The rigor develops at the same rate as the ATP disappears. At the maximum of rigor there is no ATP at all. (Fig. 1.)

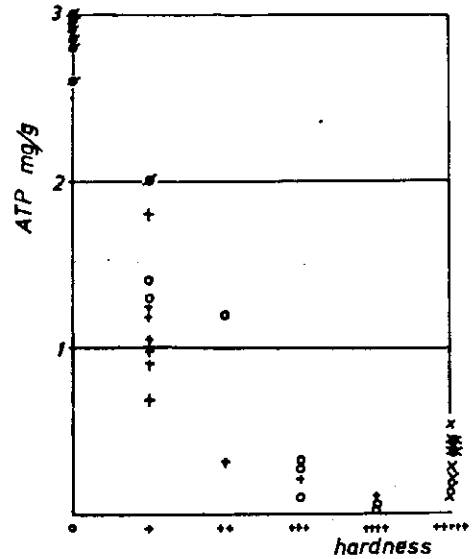


Fig. 2. ATP content and hardness of frog muscle, in different forms of contractures

0 is the hardness of fresh muscle.
+++++ is the hardness of muscle in maximal rigor.
⌀ Fresh muscle.
○ Muscle 24—48 hours after decapitation. (At 18—20°)
+ Electrically stimulated muscle.
× Isolated gastrocnemius exposed to chloroform vapours for 12 mins.
● Left leg
● Right leg, stimulated of frog poisoned with iodoacetate.

The hardness was measured *in situ*. The same muscles were used for ATP determination.

Parallel with the development of the rigor the myosin becomes more and more insoluble showing that both phenomena, rigor and insolubility are closely connected. By addition of ATP the solubility of myosin could be restored. This supports the conclusion that both the rigor and the decreased

solubility were, in fact, due to the reduction of the ATP concentration. In absence of ATP, myosin is practically insoluble.

Parallel to the relaxation of the rigor the myosin becomes soluble again and goes into solution even without addition of ATP. In this case, however, it is not myosin that goes into solution but actomyosin. It can be concluded that the relaxation of rigor mortis is thus due to desorganisation, i. e. to the release of actin from the structure.

In the other forms of rigor there is less close parallelism between contracture and ATP concentration than in the case of rigor mortis. (Fig. 2.)

**Experimental part.**

The hardness of the muscle was measured by MANGOLD'S[6] method. I worked throughout with 10 g weight.

Myosin was extracted with 3 ml 0,6 *M* KCl pro g muscle. The muscle was minced in the Latapie mincer, suspended in the KCl solution, stirred for ten minutes and centrifuged. In order to estimate the myosin content of this extract 1 ml. 0,5 *M*, pH 5,2 acetate buffer and 8 ml water were added to 1 ml of it. The precipitate was centrifuged off, washed twice, dried and weighed.

The ATP was estimated in extracts of muscle according to SZENT-GYÖRGYI (see page 93.).

Rigor mortis: The hardness was measured on one and the same place of the thigh of the decapitated and skinned rabbit. From the other thigh I excised, after certain periods, 10—10 g samples. 3 g were used to estimate the solubility of myosin without addition, 3 g with addition of ATP. (3 mg ATP was added pro g. muscle). In 3 g of the muscle the ATP was estimated. The results are summed up in Table 1. The data of the first series of experiments are shown also in Fig. 1, where the correlation between the different factors can be seen.

I was unable to pull threads from the myosin extracted from the muscle in rigor, which shows that there was very little actin present. The myosin extracted after relaxation can readily be pulled to threads. It was a 9% actomyosin and contracted on addition of K, Mg and ATP: 66%.

In the case of monoiodoacetic acid, and caffeine I injected the solutions into the lymphsac of the frog. (0.4 mg iodoacetate

*Table I.*

| Hours | Hardness % | Soluble myosin mg/ml | Soluble myosin mg/ml in presence of 100 mg % ATP | ATP mg/g muscle |
|---|---|---|---|---|
| 0 | 0 | 18 | 18 | 3 |
| 3 | 36 | 17 | 17.5 | 2.2 |
| 6 | 60 | 16 | 18 | 1.4 |
| 8 | 86 | 10 | 16 | 0.4 |
| 10 | 96 | 4 | 11.5 | 0 |
| 12 | 100 | 4 | 11 | |
| 18 | 100 | 4 | 11 | |
| 24 | 86 | 5 | 12 | |
| 48 | 43 | 16 | 17 | |
| 0 | 0 | 14 | 15 | 2.8 |
| 5 | 50 | 15 | 15 | 1.4 |
| 6 | 70 | 10 | 15 | 0.7 |
| 9 | 100 | 4 | 13 | 0 |
| 0 | 0 | 13 | 15 | 3 |
| 6 | 50 | 13 | 16 | 1.1 |
| 24 | 100 | 3 | 16 | 0 |
| 0 | 0 | 18 | 18 | 2.9 |
| 6 | 100 | 4 | 17 | 0 |
| 0 | 0 | 16 | 17 | |
| 10 days At 0° | 40 | 14 | 14 | |
| 0 | 0 | 15 | 16 | |
| 8 days At 0° | 45 | 14 | 15 | |

The hardness of fresh muscle: 0%.
Rigor mortis: 100%.

per g, 0,15 mg caffeine per g). After the rigor had developed I estimated the solubility of myosin with and without the addition of ATP. The results are summed up in Tab. 2. In one experiment the lower half of the spinal cord, innervating the lower limb, was destroyed. When rigor began to develop in the fore limb I stimulated one hind limb electrically for a minute and then used both limbs for estimation of rigor and ATP. The result of this experiment is summed up with other experiments in Fig. 2.

*Table II.*

| Soluble myosin mg/ml extracted with : | | | | | |
|---|---|---|---|---|---|
| 3 ml 0.6 *M* KCl/g muscle | | | 3 ml 0.6 *M* KCl/g muscle in presence of 100 mg % ATP | | |
| Fresh resting muscle | Muscle in iodoacetate rigor | Muscle in caffeine rigor | Fresh resting muscle | Muscle in iodoacetate rigor | Muscle in caffeine rigor |
| 7 | 1.1 | 5 | 8 | 8.5 | 11 |
| 11 | 2.5 | 5.5 | 12 | 11 | 12 |
| 11.1 | 4 | | 11 | 11 | |
| 9.5 | 3.6 | | 10 | 9.2 | |
| 10 | 5 | | 11 | 22 | |

The ATP content of muscles in rigor was 0.10—0.20 mg/g.

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