THE CHANGE IN STATE OF THE PROTEINS OF MUSCLE 
IN RIGOR

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When a muscle contracts or goes into rigor, the myosin within it becomes changed, the change being detectable as a loss in solubility. The difference between the state of myosin in rigor and the state of myosin which has been rendered insoluble by a typical denaturing agent, such as heat or acid, is described in this paper.

Although the altered solubility of muscle proteins in rigor was observed 27 years ago by Saxl, it has been the subject of very few investigations. Saxl thought that the change in solubility was in the myogen (albumin fraction) of muscle, and it was not until 1924 (Howe), or more decisively until 1933 (Weber and Meyer) that this mistake was corrected and it was shown that the change was in myosin. In 1930 Deuticke observed that a similar, or perhaps identical, change occurs in myosin during contraction, and that on recovery the change is reversed. By loss of solubility, it is not meant that myosin precipitates within the muscle. There is indeed evidence that even in resting muscle myosin is not in solution (Smith, 1934). The myosin of active muscle or of muscle in rigor is modified so that when the structure of the tissue is destroyed this protein does not dissolve in a salt solution in which the myosin of resting muscle does dissolve. The scale of the phenomenon is striking; in rigor 30 per cent of the total protein in muscle becomes changed.

Many proteins are modified when exposed to heat, acid, alkali, alcohol, urea, salicylate, ultraviolet light, surface forces, or other agents so that they are no longer soluble under conditions under which the unchanged proteins are soluble. The change is known as denaturation. Because of the superficial resemblance, the change in solubility that occurs in myosin has also been referred to as "denatura-
tion (Weber and Meyer, von Baeyer and von Muralt). It is now possible to define denaturation more precisely than could be done formerly (Mirsky and Anson, 1935–36). When a protein is acted upon by any one of the agents just mentioned, there occurs both a loss of solubility and an activation of all its S-S and SH groups. The two changes are so closely linked that they must be considered to be integral parts of the same process. Denaturation has hitherto been defined by the change in solubility alone, but since all known denaturing agents activate S-S and SH groups at the same time that they render the protein insoluble, denaturation is more completely and precisely defined in terms of both of these changes.

When myosin is treated with a reagent that denatures other proteins the changes characteristic of denaturation are observed. But when in rigor myosin loses its solubility it is shown by the experiments described in this paper that no activation of SH groups occurs. This change in myosin is, therefore, distinctly different from the change in it caused by a typical denaturing agent. The difference is clearly emphasized when myosin rendered insoluble in rigor is treated with a denaturing agent, acid. No change in solubility is now observed, but the SH groups of myosin are activated just as they are when native soluble myosin or any other native protein is treated with an excess of acid. Protein coagulation, loss of solubility, can therefore occur in two different ways.

Coagulation of protein in which loss of solubility occurs without the activation of SH and S-S groups characteristic of denaturation is not limited to muscle. This change can be observed in the proteins of other tissues under certain conditions to be described in another paper.

Observations of the SH groups of myosin are useful in interpreting some recent experiments of Astbury and Dickinson on the crystallographic properties of myosin. In his studies of the crystal pattern of wool, as revealed by the x-ray diffraction method, Astbury finds that certain changes occur when wool is treated with steam. When myosin is exposed to steam similar changes are detected. Astbury and Dickinson suggest that the myosin in muscle undergoes this change in the course of muscular activity. When a fiber of myosin, such as was used by Astbury and Dickinson is exposed to steam for only 2 seconds, I find that all its SH groups have been activated, that the
myosin has been denatured. The change in myosin known to occur when muscle becomes active is therefore distinctly different from the change that Astbury and Dickinson suppose takes place. If a change in myosin like that due to steam occurs at all in muscle, it occurs in such a minute quantity of myosin that it is not detectable by the present methods for estimating protein SH groups.

**EXPERIMENTAL**

The experiments were on the skeletal muscle of the southern bull frog (*Rana catesbiana*). Rigor was produced, as described by Lundsgaard, by the injection of iodoacetate in a dorsal lymph sac. 10 cc. of m/10 iodoacetate (iodoacetic acid neutralized with sodium hydroxide) were injected. The onset of rigor became apparent in 2 or 3 hours, and the frog was then left in a cold room at 8° overnight. Next morning its limbs were stiff.

The muscles of the hind leg were minced, and the number of active SH groups was estimated by the reaction with iodoacetate in precisely the same manner previously described for minced muscle prepared from resting muscle. The procedure for denaturing the proteins in minced rigor muscle is the same as that previously described for resting muscle.

The quantity of protein becoming insoluble in rigor was measured. Muscles at rest and in rigor were minced, and 15 gm. of each were transferred to 250 cc. centrifuge flasks. To each flask were added 200 cc. of cold 1.2 m KCl and 4 cc. m/1 KHPO₄. The flasks were placed in ice mixtures and their contents stirred for 2 hours. After centrifuging, the supernatant fluid of each flask was poured into a 500 cc. flask, and to the residue in each centrifuge flask were added 225 cc. 1.2 m KCl, in which the minced muscle was re-extracted for 2 hours. To the total supernatant fluid of each extraction were added 30 cc. concentrated trichloracetic acid (the acid dissolved in an equal weight of water) to precipitate the protein. This precipitate and the residue of extracted tissue, both in 250 cc. centrifuge flasks, were freed of salt by repeated washing with 5 per cent trichloracetic acid, the washing fluid being removed by centrifuging. Each precipitate, in a 250 cc. centrifuge flask, was dehydrated by washing twice with acid-acetone (1 cc. of concentrated HCl in 200 cc. of acetone), and the lipoids which remained were removed by washing twice with a mixture containing three parts of alcohol and one part of ether. The dry weight of the substance in each flask, which now consisted practically entirely of protein, was determined after drying to constant weight at 110°.

Fibers of frog myosin spread out in a thin sheet were exposed to steam for 2 seconds. On testing with nitroprusside and ammonium hydroxide an intense reaction was obtained indicating the presence of SH groups. The steamed fibers were treated with iodoacetate at pH 7.3 for 3 hours and were then found to give no test for SH groups. The preparation was completely denatured, for even after subsequent treatment with trichloracetic acid it failed to give a test for
PROTEINS OF MUSCLE IN RIGOR

SH groups. When a myosin fiber, which has not been exposed to steam is treated with iodoacetate and then with trichloracetic acid an intense test for SH groups is obtained. After treating with trichloracetic acid it is important to wash the protein with 1/2 pH 7.3 phosphate buffer before testing with nitroprusside.

RESULTS

Extraction of Protein from 15 Gm. of Minced Muscle

<table>
<thead>
<tr>
<th></th>
<th>Resting muscle</th>
<th>Muscle in rigor</th>
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</thead>
<tbody>
<tr>
<td>Weight of protein extracted</td>
<td>1.94 gm.</td>
<td>1.0 gm.</td>
</tr>
<tr>
<td>Weight of residual protein</td>
<td>0.93</td>
<td>1.61</td>
</tr>
<tr>
<td>Total protein</td>
<td>2.87</td>
<td>2.61</td>
</tr>
<tr>
<td>Percentage of protein extracted</td>
<td>67.6</td>
<td>38.3</td>
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</tbody>
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SH Groups of the Proteins in Minced Muscle

Muscle in rigor—I
1. Cysteine content of untreated muscle proteins... 0.74 per cent
2. Cysteine content after treating with iodoacetate at pH 7.3... 0.62 per cent
3. Percentage of total number of SH groups that react with iodoacetate, that is (1)-(2) over (1)...... 15.8 per cent

Muscle in rigor—II
1. Cysteine content of untreated muscle proteins... 0.75 per cent
2. Cysteine content after treating with iodoacetate at pH 7.6... 0.62 per cent
3. Percentage of total number of SH groups that react with iodoacetate... 17.6 per cent
Average of the two experiments... 17 per cent

Resting muscle
Percentage of total number of SH groups reacting with iodoacetate at pH 7.3 and 7.6
Average of several experiments taken from a previous paper... 19 per cent

Rigor muscle denatured by trichloracetic acid
Cysteine content of mixed proteins... 0.74 per cent
Cysteine content after treating with iodoacetate at pH 7.3 ............... too low to estimate accurately
Percentage of total number of SH groups that react with iodoacetate ..................... nearly 100 per cent

CONCLUSIONS
1. When myosin is exposed to a typical denaturing agent (acid) it becomes insoluble and its SH groups are activated.
2. The same number of active SH groups is found in the soluble myosin of resting muscle as in the insoluble myosin of muscle in rigor. No activation of SH groups accompanies the formation of insoluble protein in rigor.
3. When the insoluble myosin of muscle in rigor is treated with a denaturing agent its SH groups are activated.
4. Protein coagulation as brought about by denaturing agents (heat, acid, alkali, alcohol, urea, salicylate, surface forces, ultraviolet light) is a distinctly different change from the coagulation of myosin brought about by the unknown agent in muscle.

REFERENCES