FORMATION OF LACTIC ACID, AN INITIAL PROCESS IN WORKING MUSCLE*

By EUNICE V. FLOCK, DWIGHT J. INGLE, AND JESSE L. BOLLMAN
(From the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota)

(Received for publication, March 20, 1939)

The early chemical changes in continuously contracting muscle have been definitely shown to involve anaerobic reactions. The formation of lactic acid from glycogen, the hydrolysis of phosphocreatine, the hydrolysis of adenosinetriphosphate, and the formation of hexosemonophosphate liberate energy which may be used for muscular contraction. Most of the theories of muscular contraction postulate some degree of interdependence of these reactions and an oxidative recovery phase involving the reversal of the anaerobic reactions. With continuous work the oxidative recovery of these substances is assumed to increase until the resynthesis is equal to the somewhat lessened anaerobic hydrolysis when a steady state is reached. The immediate energy of contraction is considered as being continually derived from the anaerobic processes maintained in continuous reaction by energy from oxidative processes.

Lundsgaard (1) in a recent review stated that the question might well be raised as to the extent to which the known anaerobic processes participate in the metabolism of aerobically working muscle; that is, muscle working with unimpaired circulation and adequate oxygen supply. Sacks and his collaborators (2-4), as well as others since Embden et al. (5), have seriously questioned the part played by the anaerobic processes in the contraction of muscle working aerobically and have indicated that the chemical reactions of anaerobiosis are much less definitely related than they were assumed to be. Sacks, Sacks, and Shaw (3) compared the chemical changes occurring during and after stimulation of muscle.

* Read before the Division of Biological Chemistry of the American Chemical Society at Milwaukee, Wisconsin, September 5-9, 1938.
in cats by comparing the contents of the stimulated and the corresponding unstimulated muscle. At one twitch per second the lactic acid rapidly increased to a peak in 1 minute and subsequently declined. Phosphocreatine decreased rapidly and subsequently increased slowly. No change occurred in the adenosinetriphosphate or the hexosemonophosphate content of the muscle. At two twitches per second hydrolysis of adenosinetriphosphate and synthesis of hexosemonophosphate occurred rapidly within the 1st minute and decreasingly less at subsequent times. Sacks found that in recovery from 5 minute stimulation twice each second phosphocreatine was resynthesized slowly and adenosinetriphosphate even more slowly and indicated that the rate of resynthesis is too slow to be of great importance in furnishing energy for continued muscular contraction. He concluded that the contracting muscle uses oxidative reactions directly as a source of energy. Bang (6) on the basis of analysis of the blood of human beings engaged in moderate exercise also suggested that lactic acid formation occurs only in the 1st minute of muscular work. Kramer (7) found that approximately 1 minute was required for the blood flow of resting muscle to become maximal after the onset of vigorous activity.

Our observations of the changes occurring in contracting rat muscle are in general similar in import to those of Sacks and his collaborators. In our use of a more rapid rate of stimulation the changes produced in the acting muscle are more pronounced and may be compared on a different basis. Under these conditions the major portion of change in lactic acid, glycogen, phosphocreatine, adenosinetriphosphate, and hexosemonophosphate is completed within the 1st minute of work. The rate of these changes, the subsequent alterations with continued work, and the alterations occurring with rest indicate an independence of the anaerobic reactions and their reversal. In addition, our data appear to indicate that the anaerobic reactions are involved in the initial processes of muscular work but are not greatly involved in its continuation.

EXPERIMENTAL

Male rats of the Wistar strain, which weighed from 200 to 300 gm., after a fast of 24 hours were anesthetized with amytal and the skin was loosened from the hind legs and held in place with
loose sutures. The Achilles tendon of the left leg was loaded with a weight of 100 gm. and the muscle stimulated three times each second by a direct faradic current as described by Heron, Hales, and Ingle (8). Mechanical recorders of the movement of the weight indicated a constant amount of work performed during the first 40 to 80 seconds after which time there was a decrease of about 30 per cent, which was associated with a definite decrease of muscle tonus. No further changes of the amount of movement occurred during these experiments, the recorders indicating similar activity in each succeeding minute. During periods of rest the weight was removed from the tendon. At the time indicated the gastrocnemius muscle was removed and plunged into a freezing mixture of carbon dioxide ice and alcohol. Not more than 3 seconds elapsed during this process. The unstimulated gastrocnemius muscle of the right leg was removed in the same manner.

The frozen muscle was fragmented between cooled steel blocks, quickly weighed, and dropped into tubes containing ice-cold 5 per cent trichloroacetic acid and glass beads or the hot potassium hydroxide. The tubes were shaken for 10 minutes and the solution was filtered. Because of the small quantity of muscle available, different series of rats were used for the determination of lactic acid, glycogen, and phosphate. Lactic acid was determined by the method of Friedemann, Cotonio, and Shaffer (9) after preliminary treatment with copper sulfate and calcium hydroxide glycogen by the modified Pfluger method, and phosphate by the method of Fiske and Subbarow (10). The modified Eggleton procedure for fractionation of the acid-soluble phosphates was used (11). The phosphocreatine was determined directly. The soluble ester or hexosemonophosphate was the difference between this and the entire barium-soluble fraction. The inorganic phosphate and the phosphate hydrolyzable in 15 minutes, which would represent chiefly the labile part of adenosinetriphosphate, were determined on the barium-insoluble fraction. The original extract and the two fractions thereof were ashed with sulfuric acid and superoxol. The values reported are differences between the stimulated and unstimulated muscles.

**Results**

Analyses of the muscle from both hind legs of the resting rat showed but small differences in the contents of each gastrocnemius
Formation of Lactic Acid

muscle. The concentrations of the various compounds in the resting muscle of the experimental animals showed a much greater variation which did not appear to be correlated with the changes found in the stimulated muscle. Such variations, however, were small in comparison to the changes in the stimulated muscle and were probably due to differences in the depth of anesthesia and in the amount of manipulation involved. The content found in the resting muscle of the various experiments was lactic acid 15.6 ± 3.8 mg. (s.d.), per 100 gm. of muscle, and phosphocreatine 45.4 ± 6.0, adenosinetriphosphate 34.0 ± 4.5, hexosemonophosphate 11.2 ± 4.0, inorganic phosphate 28.6 ± 6.0, each expressed as mg. of phosphorus per 100 gm. of muscle.

Tabulation of the differences in the concentration of the various compounds found in the stimulated and unstimulated muscle of each animal after a definite period of stimulation showed the fol-

Fig. 1. The decrease in glycogen, expressed as the difference in the glycogen content of the stimulated and the unstimulated muscle on the opposite side of the same animal. The stimulated muscle contracted three times each second for the entire period of time indicated on the abscissa.
lowing changes to be present in the intermittently stimulated muscle. The glycogen content rapidly decreases progressively during the 1st minute; after this time there is little significant further change (Fig. 1). The lactic acid content increases progressively during the 1st minute; after this time there is a much slower decrease until resting values are present after approximately 15 minutes (Fig. 2). These values remain unchanged with the continuance of work. The content of phosphocreatine also decreases during the 1st minute and there is little further change as work continues (Fig. 3). The content of adenosinetriphosphate shows little change during the first 20 to 30 seconds, decreases rapidly during the next 30 to 40 seconds and then shows some tendency to increase slowly as work continues for 30 to 60 minutes, but does not return to resting values as long as work is continued. The content of hexosemonophosphate increases rapidly during the 1st minute; after this time there is a slow decrease until

![Graph showing changes in lactic acid content](http://www.jbc.org/)

**Fig. 2.** The increase in lactic acid, expressed as the difference between the lactic acid content of the stimulated and unstimulated muscle from the opposite side of the same animal. In the groups represented by ●, X, and ◇, the stimulated muscle contracted three times each second for the entire period of time indicated on the abscissa. In the group represented by ○, stimulation was continued only for 1 minute and the subsequent period of time indicated on the abscissa represents a period of rest.
Formation of Lactic Acid

<table>
<thead>
<tr>
<th>Increase in inorganic phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Normal rats</td>
</tr>
<tr>
<td>• Hepatectomized rats</td>
</tr>
<tr>
<td>• Normal rats - 1 min. Work and Rest</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Increase in hexose phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg P per 100 gm muscle</td>
</tr>
<tr>
<td>0 10 20 30 40 50 1 5 10 15 20 25 30 35 40 45 50 55 60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decrease in phosphocreatine P</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg P per 100 gm muscle</td>
</tr>
<tr>
<td>0 10 20 30 40 50 1 5 10 15 20 25 30 35 40 45 50 55 60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Decrease in labile P (Adenosine triphosphate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg P per 100 gm muscle</td>
</tr>
<tr>
<td>0 10 20 30 40 50 1 5 10 15 20 25 30 35 40 45 50 55 60</td>
</tr>
</tbody>
</table>

FIG. 3. The changes in concentration of phosphate compounds during work. The changes in phosphate compounds are expressed as differences in content in the stimulated and the unstimulated muscle on the opposite
approximately 15 minutes, when resting values are present. These values remain unchanged as work continues. The content of inorganic phosphate increases rapidly during the 1st minute and remains elevated as long as work continues (Fig. 4).

Similar tabulation of the differences found in the concentration of the various compounds in the stimulated and unstimulated muscle after definite periods of rest following stimulation for 1 minute showed the following changes to be present in the previously stimulated muscle. The glycogen content remained low, that is at the level usually found after 1 minute of stimulation, and showed no tendency to increase with rest of as much as 1 hour. The lactic acid content gradually decreased until resting values were present after about 15 minutes of rest and no further change occurred (Fig. 2). The content of hexosemonophosphate also

side of the same animal. The stimulated muscle contracted three times each second for the entire period of time indicated on the abscissa. The concentration is expressed as mg. of phosphorus per 100 gm. of muscle. In the group represented by O, stimulation was only for 1 minute and the subsequent period of time indicated on the abscissa represents a period of rest.

---

**Figure 4**: A composite picture of the changes occurring in muscle (expressed as mg. of each substance per 100 gm. of muscle) during continuous contraction at the rate of three times each second. Each point represents an average value.
Formation of Lactic Acid

decreased until resting values were reached after 15 minutes of rest. The changes in glycogen, lactic acid, and hexosemonophosphate appeared identical whether work was continued or rest ensued. The adenosinetriphosphate content gradually increased so that resting values were obtained after about 15 minutes of rest, which is different from the much slower return to resting values as found with continuous work. There is a rapid increase in the phosphocreatine content so that values almost equivalent to the resting value are found within 3 to 5 minutes of rest. The inorganic phosphate content of the muscle decreases rapidly so that resting values are found within 2 to 5 minutes after the cessation of work. This is in marked contrast to the continued low values of phosphocreatine and high values of inorganic phosphate found with continued work (Fig. 3).

Muscle stimulated for 1 minute and allowed to rest for 1 or more minutes and then restimulated for 1 minute showed changes of the same nature as those found after 1 minute stimulation alone (Table I). When these changes are considered in relation to the values for the different compounds expected to be present at the moment of the beginning of the last period of stimulation, it appears that the changes have been less in amount than would occur with the initial 1 minute stimulation but have been in the same direction.

**Table I**

*Production of Lactic Acid (Work-Rest-Work)*

<table>
<thead>
<tr>
<th>Time of rest</th>
<th>Lactic acid</th>
<th>Time of rest</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>mg. per 100 gm. muscle</td>
<td>min.</td>
<td>mg. per 100 gm. muscle</td>
</tr>
<tr>
<td>3</td>
<td>171</td>
<td>13</td>
<td>137</td>
</tr>
<tr>
<td>4</td>
<td>122</td>
<td>18</td>
<td>111</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>18</td>
<td>106</td>
</tr>
<tr>
<td>8</td>
<td>141</td>
<td>28</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>92</td>
<td>28</td>
<td>93</td>
</tr>
</tbody>
</table>

* Leg stimulated for 1 minute, rested the specified time, then stimulated again for 1 minute. The value for the lactic acid is expressed as the difference in lactic acid content between the working muscle and the unstimulated muscle. These differences are definitely less than are found with the first 1 minute stimulation but definitely more than would be present with continuous stimulation as shown in Fig. 1.
Stimulated muscle of heptatectomized rats in which the concentration of blood sugar was low brought about changes in the concentration of the various substances of the muscle very similar to those found in intact rats after similar stimulation. Adrenalectomized rats, many of which were in such a state of insufficiency that the muscle was completely fatigued after short periods of stimulation, showed changes in the stimulated muscle similar to normal animals at intervals of continuous work and also after intervals of rest.

**Comment**

Analyses of muscle made in the course of our experiments showed consistent differences between the stimulated and the non-stimulated muscle at corresponding intervals of time. The absolute values found, however, may not be taken directly as the amount of these substances formed or destroyed during the time intervals involved. Diffusion into the blood and lymph with the increased amount of blood flow induced by exercise as well as the various processes of destruction, oxidation, and resynthesis may alter the total amount of the substances present. All of our experiments were conducted under amytal anesthesia, which might induce changes not found in the unanesthetized animal. No major process appears to be involved, however, because muscular contractions may be maintained continuously and unaltered in intensity for several days.

It is interesting to note that the labile substances studied, namely glycogen, phosphocreatine, and adenosinetriphosphate, all break down under the conditions of our experiments during the 1st minute of work and work may then continue for some time without further change in the concentration of these substances. Glycogen and phosphocreatine break down simultaneously but adenosinetriphosphate breaks down more slowly. This is contrary to the idea of Lohmann (12), which is still advocated by Lundsgaard (1) and by Needham (13) and which was derived from a study of cell-free muscle extract, that the breakdown of adenosinetriphosphate precedes that of glycogen and that energy for its resynthesis may then be supplied by hydrolysis of phosphocreatine and glycogen as well as by oxidation.

Among the products of breakdown of these three labile sub-
stances are lactic acid, hexosemonophosphate, and inorganic phosphate, all of which reach maximal concentrations during the 1st minute of work. The concentration of inorganic phosphate remains elevated as work continues but the concentrations of lactic acid and hexosemonophosphate return to normal within 15 minutes. That these two substances may parallel each other in their rates of formation and recovery has been previously shown by Sacks, Sacks, and Shaw (3). We were unable to detect any formation of the labile hexose-1-monophosphate described by Cori and Cori (14).

It would appear that the removal of lactic acid, which accumulates in the muscle during the 1st minute of work and the excess of which is completely removed during the next 15 minutes, is accomplished by diffusion into the blood and little if any by increased oxidation or resynthesis into glycogen. If increased oxidation of lactic acid were much of a factor, the lactic acid formed on restimulation after a few minutes rest should be more rapidly removed. Stimulation at this time, when the lactic acid content of the muscle is rapidly decreasing, produces an increase in the lactic acid content of the muscle. For similar reasons, rapid formation of lactic acid may be said to cease after the 1st minute of exercise in our experiments. The rate of its decrease to resting values is the same with continued work or rest or after the increase with subsequent work. If it were formed continuously with work, the diminution of its content in the muscle would indicate an increased rate of its removal which should be made apparent by a more rapid removal immediately following cessation of work or during a subsequent period of work.

Phosphocreatine, like glycogen, remained at a low level as work continued but unlike glycogen was rapidly resynthesized if rest ensued. The changes observed in the inorganic phosphate content of the muscle were synchronous with the changes in phosphocreatine but in the opposite direction. In the past phosphocreatine changes have been considered both as the cause and effect of the production of lactic acid from glycogen. Thus Lundsgaard (15) in 1931 proposed the idea that glycogen breaks down to supply energy for the resynthesis of phosphocreatine and Meyerhof (16) in 1937 still accepted this idea. Fiske and Subbarow (17) considered that the function of the hydrolysis of
phosphocreatine is to supply alkali for the neutralization of the
lactic acid formed from glycogen. The Sacks, who agree with this
idea, found that in the recovery phase the phosphocreatine is
resynthesized in amounts equivalent to the quantity of lactic acid
removed. In our work the recovery of phosphocreatine took
place only during rest, whereas the recovery of lactic acid occurred
at the same rate during work or rest. Work can continue in the
normal animal without any apparent formation of lactic acid and
with a low concentration of phosphocreatine in the muscle, a
situation which is strikingly similar to that found in iodoacetate-
poisoned animals in anaerobiosis by Lundsgaard. Lundsgaard
postulated that in the presence of oxygen the phosphocreatine
would be restored to normal by oxidative resynthesis, and Bang
(6) and Bugnard (18) also assumed that this mechanism is utilized
during aerobic muscular activity when lactic acid is not being
formed. However, in our experiments, although it might be
considered that the cessation of the breakdown of glycogen is due
to the rapid attainment of an adequate supply of oxygen, this
supply is still inadequate for the resynthesis of phosphocreatine.

Comparisons of the concentration of adenosinetriphosphate and
hexosemonophosphate content of the muscle at various times of
exercise and subsequent rest also appear to indicate that these
substances are mainly involved in early phases of work and par-
ticipate but little in continued work in the steady state.

Since continued aerobic muscular activity does not involve
further change in the concentrations of the substances studied, it
is concluded that other unknown substances must then be in-
volved as sources of energy. These may exist either in the muscle
or may be brought there by the blood.

SUMMARY

Analysis of rat muscle with its blood supply intact and contract-
ing at the rate of three times each second, at varying intervals of
continuous work and rest, indicated that the following changes
occur. During the 1st minute of work there is a rapid accumula-
tion of compounds in the muscle which are associated with anaer-
bic processes. The content of glycogen, phosphocreatine, and
adenosinetriphosphate decreases rapidly and there is a rapid
increase of lactic acid, inorganic phosphate, and hexosemono-
phosphate. The subsequent changes that occur with continued work, rest, or rest followed by additional work are such that it appears that each of these chemical processes is in part independent of the other, and is little involved in the maintenance of continuous work. The postulation that muscle working in a steady state derives its energy directly from substances other than these appears necessary.

BIBLIOGRAPHY

FORMATION OF LACTIC ACID, AN INITIAL PROCESS IN WORKING MUSCLE
Eunice V. Flock, Dwight J. Ingle and Jesse L. Bollman


Access the most updated version of this article at http://www.jbc.org/content/129/1/99.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/129/1/99.citation.full.html#ref-list-1