5494

## Phosphate Changes in Chloroform Rigor With and Without Production of Lactic Acid.

ETHEL RONZONI. (Introduced by P. A. Shaffer.)

*From the Laboratory of Biological Chemistry, Washington University School of Medicine.*

The new technic of Lundsgaard[1] for studying the chemical changes occurring in muscle during activity gives an opportunity for a new approach to the relationship between contraction and rigor. We have, therefore, studied the effect of mono-iodoacetate on the chemical processes of chloroform rigor, since the changes occurring in this form of rigor have been supposed to be similar to those of muscle contraction.

The muscles were soaked for 40 minutes in an oxygenated solution of Na mono-iodoacetate in Ringers (1 to 5000); one of the pair was treated with chloroform and the other analyzed for control. The control and experimental muscles were frozen at the same time, thinly sliced and extracted with trichloracetic. On this extract the various phosphate fractions and lactic acid were determined. The changes in the phosphate fractions and in lactic are in the table.

TABLE I.

*Treated Muscles.*

Values represent changes due to chloroform expressed in mg. per 100 gm.

| Muscle used | Rectus | Rectus | Sartorius | Sartorius |
|---|---|---|---|---|
| Time of Chl. | 30 | 35 | 60 | 90 |
| Lactic | +21.0 | +30.2 | ± 0 | +58.0 |
| Free P. | +47.0 | +41.8 | +30.4 | +32.9 |
| Creatine P. | —33.9 | —29.5 | —56.0 | — 8.6 |
| Pyro P. | —14.9 | —16.3 | —10.3 | — 8.3 |
| Other Esters | — 6.0 | + 3.9 | +25.9 | +16.0 |

*Untreated Muscles.*

| Muscle used | Rectus | Rectus | Gastroc | Gastroc |
|---|---|---|---|---|
| Time of Chl. | 30 | 40 | 30 | 30 |
| Lactic | 240.0 | 416.0 | 420.0 | 181.0 |
| Free P. | +20.0 | +26.1 | +44.8 | +20.3 |
| Creatine P. | —32.3 | —20.2 | —10.7 | —32.3 |
| Pyro P. | —10.5 | —13.9 | —16.3 | —11.5 |
| Other Esters | +21.5 | + 8.2 | —15.0 | + 9.5 |

[1] Lundsgaard, E., *Biochem. Z.*, 1930, **227**, 57.

The decrease in phospho-creatine is more marked in the poisoned muscles than in those in which lactic acid is produced. Admitting the same interpretation as for muscle contraction one must conclude that phospho-creatine is the source of energy and that its resynthesis through the lactic acid mechanism is not interfered with. The high lactic acid values in relation to the extent of phospho-creatine resynthesis show this to be a wasteful process from the energy standpoint.

Analysis made when the muscles have just reached their maximum shortening show little change in the remaining bound phosphorus (other esters in the table) but with time this fraction increases especially in muscles poisoned with mono-iodoacetate. No analysis of this fraction has been made but it is probably carbohydrate ester.

The most striking change occurring in rigor is uninfluenced by mono-iodoacetate, the breakdown of pyrophosphate-adenylic acid. The degree of correspondence between the extent of breakdown of pyrophosphate and the tension of rigor is not adequate to show a quantitative relationship. When rigor is complete 50 to 60% of the pyrophosphate has disappeared. The remainder breaks down within an hour. In mono-iodoacetate rigor, Parnas[2] and Lundsgaard[1] have shown a production of $NH_3$ accompanying the hydrolysis of pyrophosphate-adenylic acid with the formation of 1 mole of $NH_3$ to 2 of phosphate. In contraction, although $NH_3$ is liberated, presumably from pyrophosphate-adenylic acid, either the course of reaction is different or resynthesis occurs immediately, since there is little change in this phosphate fraction, unless stimulation proceeds to extreme fatigue anaerobically. The breakdown of pyrophosphate-adenylic acid appears to be the result of the processes causing rigor rather than the cause of rigor itself, unless the inosinic acid formed is more toxic than $NH_3$ or phosphate.

Iodo-acetic acid rigor occurs in muscles, after stimulation, when phospho-creatine has been completely broken down and when no lactic acid is being formed. It is therefore different from chloroform rigor, or the hydrolysis of phospho-creatine is incidental to the mechanism as is lactic acid.

[2] Parnas, J. K., *Naturwissen*, 1930, **44**, 916.