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pH Changes of Muscle During and After Contraction.

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Since Meyerhof and Lipmann,[1] and Meyerhof, Moehle and Schulz[2] studied the exchange of CO_2 in frogs' muscle during fatigue, we know that under anaerobic conditions the pH of the muscle, when subjected to periodic stimulations, will first increase and then gradually decrease. However, the method applied by these authors does not show the pH changes during the activity itself, *i. e.*, contraction and relaxation of the muscle. Margaria and Pulcher[3] attempted to show the pH change during contraction itself by coloring the muscle with bromocresol purple. They found a change in color during the contraction corresponding to an increase in pH of about 0.5. According to Margaria,[4] however, the same changes occur when the muscle is mechanically stretched. Later Margaria and von Muralt[5] described a photo-electric method with which it would be possible to record those changes of color. On summarizing, as far as I know, no records showing the pH changes during and after contraction have been published. I succeeded in obtaining such records during tetanus by an entirely different method. Its principle is to cover a part of the surface of the muscle with a glass electrode and to record the pH changes occurring on stimulation.

The glass electrode consists of a membrane of suitable glass sealed to the end of a tube of ordinary glass. This is the first of the two types of glass electrode described by MacInnes.[6] The glass membrane is brought into contact with the wet external surface of the muscle. Glass electrode, muscle, and a potentiometer are mounted in series in the grid circuit of a Pliotron tube F.P. 54.[7] A galvanometer is placed in the plate circuit. The deflections of the galvanometer light spot due to a change in the grid voltage are recorded on a silver-bromide paper camera. The inside of the glass electrode tube contains 0.1 N HCl and a silver silver-chloride electrode.[8] The

1 Lipmann, F., and Meyerhof, O., *Biochem. Z.*, 1930, **227**, 84.

2 Meyerhof, O., Möhle, W., and Schulz, W., *Biochem. Z.*, 1932, **246**, 285.

3 Margaria, R., and Pulcher, C., *Boll. soc. Biol. sper.*, 1934, **9**, 879.

4 Margaria, R., *J. Physiol.*, 1934, **82**, 496.

5 Margaria, R., and v. Muralt, A., *Naturwiss.*, 1934, **22**, 634.

6 MacInnes, D. A., and Belcher, D., *J. Am. Chem. Soc.*, 1931, **53**, 3375.

7 General Electric Co.

8 Brown, A. S., *J. Amer. Chem. Soc.*, 1934, **56**, 646.

other half cell consists of a silver silver-chloride electrode immersed in Ringer solution, which, by means of a cotton thread, is held in contact with the tendon of the muscle. The muscle is kept in a glass chamber and, as a preparatory step for the experiment, is first immersed in a buffered Ringer solution for one hour through which is bubbled steadily a gas mixture of 95% O_2+5% CO_2. Then the Ringer solution is discarded and the chamber now bubbled with a mixture of 95% N_2+5% CO_2. The glass electrode is now put in contact with the muscle in the moist chamber and the muscle is stimulated through its nerve with the aid of an induction coil. Figure 1 shows a typical record.

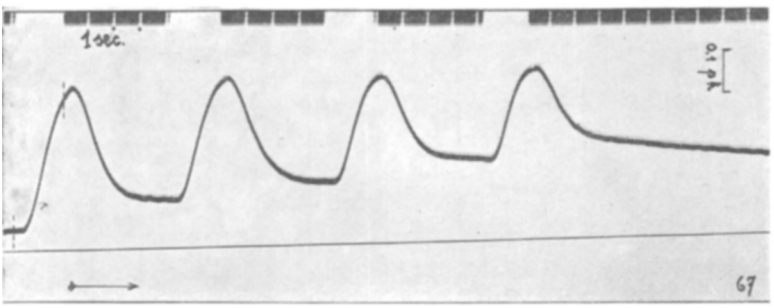

FIG. 1.
pH changes (increase up, decrease down) during and after four tetani, each of about 2 seconds, in the fresh gastrocnemius of a frog. Temperature: 25°C. At the starting point the pH was 6.91 (December 4, 1936). The small gaps in the time scale indicate seconds; the larger gaps show the periods of stimulation.

One sees that half a second after the beginning of the stimulation the thin liquid film outside the muscle, which is in contact with the glass membrane, begins to become more alkaline, gradually becoming more and more so. The maximum change is reached about half a second after the end of a stimulation and amounts to about 0.3 pH unit. After this maximum has been reached the change reverses and the film becomes gradually more and more acid. Other records have shown that one minute after a 2-second tetanus the pH is lower than it was in the beginning. The records differ according to the state of fatigue. In a nearly exhausted muscle the increase of pH during contraction is very much less, on the other hand, the decrease of pH during recovery is greater. On varying size, shape and thickness of the glass membrane it could be clearly seen that these factors have no influence on the type of the records. On the other hand it is very important to control carefully certain technical factors concerned with the exchange of CO_2. The method

is based on the fact that CO_2 freely passes across the membrane of the muscle, but the other substances involved in buffering effects are exchanged either much more slowly or not at all. For this reason this method shows no pH change at all on stimulation when the muscle has been previously freed as much as possible from CO_2 by bubbling with pure nitrogen. Furthermore, it is necessary to isolate that part of the muscle surface which is in contact with the glass membrane, from the gas mixture in the chamber. This can be easily achieved by a rubber ring. So the muscle is first brought into gas equilibrium with the gas mixture bubbling through the chamber, then the glass membrane and the rubber wall surrounding it are brought into contact with the muscle, cutting off the gas exchange between the muscle surface underneath the glass membrane and the outside gas atmosphere. Under these conditions any change in pH inside the muscle is followed, owing to the CO_2 exchange, by a pH change in the film of moisture which is situated between the glass electrode and the muscle. It is the pH change in this film of moisture due to the CO_2 exchange that we are recording with this method.

On summarizing, this method permits of the study, without any damage to the muscle fibers, of the surprisingly fast exchange of CO_2 which results from chemical processes occurring during and after contraction, between the inside of the muscle and the moist film covering its surface. The rapidity of these exchanges permits the recording of the changes of pH with relatively little lag of time (half a second). A full description of the methods and results will soon be published.

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