# The Effects of Citric Acid on Contraction of Mouse Cardiomyocytes

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**INTRODUCTION**: In the typical muscle cell bundle, there consists millions of sarcomeres, units of muscular contraction. Within those sarcomeres, three main proteins involved in muscle contraction can be found. These three proteins include actin and myosin, the two molecular components responsible for muscle contraction, as well as a third, troponin, which acts as a safeguard and prevents actin and myosin from touching by blocking off actin. Troponin is activated by an influx of calcium ions into the cell which lifts the troponin "safeguard" and allows for contraction. In the body, specifically, in the heart, this is stimulated by nerve signals from the Purkinje fibers. The membranes of such cells have calcium channels and pump proteins to regulate contractions. By adding ethylenediaminetetraacetic acid, or EDTA to the cell, the membrane becomes more permeable, allowing for calcium ions to slip past, resulting in contractions. As for the energy source of the cells, since cells require insulin to uptake glucose, we can use an intermediate of the process of cellular respiration, such as citric acid, to bypass this process, instead, diffusing the smaller molecules across the membranes of the cell and mitochondria.

**ABSTRACT**: The intention of this experiment was to provide the compound for an intermediate step in cellular respiration and allow for the mitochondria in the myocytes to produce their own ATP for muscle contraction. Both whole mouse hearts and isolated cardiomyocytes were used in this experiment. Two buffers, a control and an experimental one, were made, with the control buffer lacking citric acid and sodium citrate. For the isolated cardiomyocytes, they were bathed in 5 mL of each respective buffer, and then analyzed under a microscope to look for contractions. They were left in their respective buffers and checked every 10 minutes for 3 hours to see how long the cells would continue contracting. When using the whole heart, a Langendorff apparatus was used to perfuse the heart with the buffer, and then timed to measure how long the cells would continue contracting.

In muscular contraction, myocytes in a body make ATP from the uptaken glucose for energy. In this experiment however, instead of using ATP or glucose, we used citric acid. The intention of this experiment was to utilize a carbon intermediate in cellular respiration, specifically the Kreb's cycle, and allow for the mitochondria in cardiomyocytes to produce their own ATP for muscle contraction. Successful usage of a more readily available carbon intermediate to spur ATP synthesis with the addition of EDTA, a chelate to increase membrane permeability, would allow for live mouse cells and cardiac tissues to function without a constant source of glucose, insulin, or ATP. Both whole mouse hearts and isolated cardiomyocytes were used in this experiment. Two buffers, a control and an experimental were made, with the control buffer lacking citric acid and sodium citrate. The cells were bathed in and the whole heart was perfused with the buffers. The hypothesized result was contraction of only the cardiac tissue and myocytes treated with the citrate buffer; this result was observed in the cell samples, whereas in the tissue, both control and experimental whole mouse hearts contracted. . Since the cell samples reflected the hypothesized results, it can be inferred that citric acid was able to indirectly supply live cells with the ATP needed to contract. Furthermore, tissue density and lack

of proper distribution of the buffers may have been the reason for the whole heart contractions to occur in both samples and for a brief period of time: these contractions were likely caused by residual ATP and glucose.

**HYPOTHESIS**: Using EDTA to increase the permeability of the cell membrane, and an intermediate metabolic compound, citrate, to generate ATP, we can stimulate a mouse heart to contract without the use of electrical stimulation.

**EXPERIMENTAL GROUPS**:

**Cardiomyocytes**:

Control: control buffer (without intermediate compound, just glucose)

Experimental: citrate buffer (with intermediate compound and glucose)

**Whole heart**:

Control: control buffer (without intermediate compound, just glucose)

Experimental: citrate buffer (with intermediate compound and glucose)

**METHODS** :

**Prep buffers:**

| *"Citrate Buffer" (1x)* | *mM* | *500ml* |
|---|---|---|
| EDTA (292.24) | 3 | 0.438 g |
| Glucose (180.16) | 20 | 1.800 g |
| Tris Buffer (121.14) | 10 | 0.606 g |
| KCl (74.55) | 140 | 5.219 g |
| $MgCl_2$ hydrate (95.21) | 1 | 0.0476 g |
| $CaCl_2$ (110.98) | 2.85 | 0.158 g |
| Citric Acid (192.124) | 10 | 0.190g |
| Sodium Citrate (258.06) | 0.15 | 0.019g |

Filter with vacuum filter

Bubble Citrate buffer for 20 minutes with 95% $O_2$/5% $CO_2$

Adjust pH to 6.5-7.0 with KOH

| *"Control Buffer" (1x)* | *mM* | *500ml* |
|---|---|---|
| EDTA (292.24) | 3 | 0.438 g |
| Glucose (180.16) | 20 | 1.800 g |
| Tris Buffer (121.14) | 10 | 0.606 g |
| KCl (74.55) | 140 | 5.219 g |
| $MgCl_2$ (95.21) | 1 | 0.0476 g |
| $CaCl_2$ (110.98) | 2.85 | 0.158 g |

Filter with vacuum filter

Bubble Citrate buffer for 20 minutes with 95% $O_2$/5% $CO_2$
Adjust pH to 6.5-7.0 with KOH

Inject mouse with 100$\mu$l heparin (~20 minutes)
Inject mouse with 100$\mu$l pentobarbital (~5-10 minutes until mouse is anesthetized)
Pinch toe to confirm that the mouse is anesthetized
Cut open chest and cut out heart
Transfer heart to ice-cold PBS (keep on ice)
Clean off and cannulate aorta
Mount on Langendorf setup

For whole heart:
Perfuse with citrate buffer/control buffer on Langendorf apparatus to stimulate contractions

For cardiomyocytes:
Perfuse with Tyrode's to wash out blood (~5 minutes)
Tyrode's/enzyme solution to digest (~5 minutes, until increase in flow rate)
Keep track of flow rate to prevent overdigestion
KB solution to wash out enzyme (~5 minutes, until no longer yellow)
Cut off heart and transfer to KB on ice
Cut open heart from inside using forceps (don't use scissors)
Gently use forceps to release cardiomyocytes (check under microscope)
Filter cells using cell strainer

Split cells into two 15 mL tubes and centrifuge
Pour out KB supernatant
Dissolve in 1 mL of respective buffer and take 1mL to deposit into the plate
Add 4 mL more of respective buffers onto 60 mm plates
Look under a microscope and zoom in to particular cells.

**RESULTS**:

*For cardiomyocyte isolation*:

- **Experiment 1**: (based directly on protocol, 8/7/19) Petri dish with Control Buffer did not have any contracting cells. Citrate Buffer did have contracting cells. However, due to the centrifugation, the majority of the cells were permanently coiled up and were not the original shape. Because of this, many cells were unable to contract and respond to the buffer compounds. Those few cells that were able to contract, were of a rectangular, flat shape, with striations down the cell, whereas those irreversibly coiled cells were round and striations were not visible. Eventually, when the cells stopped, (presumably due to the lack of oxygen, denaturation of proteins, or some other unknown factor), they often became contracted fully, pulling into a dense square or oval shape. Overall, the citrate buffer did indeed follow the hypothesized outcome.

- **Experiment 2**: Due to the damaging effects of the centrifuge on live cardiomyocyte cells, I plan to transfer the cells immediately to their respective buffers instead of transfering to KB solution beforehand. In this way, the cells will not become coiled up before they've had a chance to contract.

*For whole mouse heart*:

- **Experiment 1**: Two whole live mouse hearts were used for this experiment, each on a Langendorf apparatus, and perfused with their respective buffers. Both produced visible contractions of the atria but not in the ventricular chambers. This is possibly because the ventricular myocardium proved too thick for the buffers to properly diffuse. Both resumed contractions for several minutes before stopping, possibly as leftover blood was coagulating and preventing proper diffusion of buffer solution. Though contractions were stimulated, since the result occurred in both without sufficient difference, this does not follow the hypothesized outcome.

**CONCLUSION:**

In stimulating the contraction of cardiac tissue, several known factors are required: calcium ions and ATP. As the adenosine triphosphate compound is difficult to synthesize in large quantities and is unstable, we propose the interruption of the citric acid cycle with the introduction of citric acid in the form of citrate in order to stimulate the myocytes' own mitochondria to continually produce its own ATP. This requires efficient diffusion of buffers into the cells which was more readily achieved with single-cell experiments. Thus, a modified hypothesis needs to include this factor.

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