Muscle Contraction Investigation

**Purpose:** To determine the effects of ions and ATP on muscle contraction.

**Hypothesis:** Please write a statement as to which solution will generate the greatest shortening/contraction in fiber length and why.

**Materials:**
1. Glycerinated skeletal muscle in a tube of 50% glycerol
2. Dropper vials containing the following solutions:
   a. 0.25% ATP
   b. 0.25% ATP plus 0.05% M KCl plus 0.001 M MgCl\(_2\) solution (aka. Salts or Ions)
   c. 0.05 M KCl plus 0.001 M MgCl\(_2\) (aka. Salts or Ions)
3. Sharp scissors
4. T-pins and forceps
5. Dropper
6. Petri dish
7. Microscope slides and cover slips
8. Transparent millimeter scale
9. 1 compound and 1 dissecting microscope

**NOTE:**
1. Glassware and dissecting tools should be cleaned thoroughly and well rinsed in distilled water before use!!
2. Care must be taken to avoid cross contamination between ATP and salt solutions.

**Procedure:**
1. Obtain a 1 cm length muscle bundle. Drop put it into distilled water in a petri dish. One piece is sufficient for each group of two students per one test solution.

2. Tease the segment (1cm piece) with T-pins into very thin groups of myofibers(muscle cells). Single fibers will demonstrate the greatest contraction. Strands of muscle exceeding 0.2 mm in cross-sectional diameter must not be used.

3. Mount one strand on a clean slide with a cover slip- no chemical solution added. Examine under low then high magnification using a compound microscope. Note the striations and the smooth membrane (sarcolemma). NOTE: this is your control drawing it is performed only once! * Make a labeled, detailed drawing. (include: sarcolemma, myofilaments [A bands & I bands], and nuclei – if possible).

4. Transfer 3 new thin strands to a clean microscope slide without a cover slip. Position the strands straight and parallel to each other. (Try to obtain strands of the same length!)

5. Place the slide under a dissecting microscope and measure the length(mm) of the myofibers with a transparent ruler held under the slide. Record the average length before.

6. While observing under a dissecting microscope, flood the fibers with several drops of solution containing ATP alone, ATP plus salts or ions solution.
7. After 30 sec. or more, re-measure the fibers. Record the **average length after** adding the solution.

8. Cover the same three strands **with a cover slip**. Examine under a **compound microscope**. * Make a labeled, detailed drawing, (include: sarcolemma, myofilaments [ A bands & I bands ]).

9. Repeat steps 4 through 8 two more times using clean slides, a new set of myofibers each time with the remaining two test solutions.

**Data:** Average myofiber lengths before and after adding each of the 3 solutions.  
**Note:** There should be a total of 4 labeled, detailed drawings, (include: sarcolemma, myofilaments [ A bands & I bands ], and nuclei – if possible).

**Analysis:**
1. For each of the 3 solutions, calculate the **overall average % change** in myofiber length(+/-).

\[
\text{Average \% \ change} = \frac{\text{Absolute value between average length before and after}}{\text{Larger average length value}} \times 100
\]

2. **Graph** (histogram) the overall average change in lengths before and after with **percent change depicted** for each of the 3 solutions. (use calculations above). You must have labeled axis with a legend and a title.

**Conclusion:**
1. Describe the microscopic appearance of the myofibers in each of the 3 solutions compared to those seen in step 4. (i.e. describe the appearance of I & A bands)  
2. Based on your data, which solution best facilitated a muscle contraction and why?  
3. Based on what you know, what chemical components should be present in a myofibers sarcoplasm to initiate a contraction?  
4. What **component** of a myofiber **changed in length** during a muscle contraction?  
5. What anatomical structures of a myofiber give it its striated appearance?  
6. What occurs to the H - zone of a sarcomere during a muscle contraction?  
7. What is the physical state of the cross bridges (myosin-heads) in a skeletal muscle when its internal ATP stores are exhausted? (Discuss the myosin with respect to actin.)  
8. What is the physical state of the cross bridges (myosin-heads) in a skeletal muscle when ATP is present, but there is **no free calcium**? (Discuss the myosin with respect to actin.)

**Sources of Error:**
1. List the possible sources of error relative to your experiment.  
2. Discuss the effects of these errors on the results and indicate which error causes the greatest deviation (variability).

**Hypothesis Conclusion:** Restate your hypothesis, and accept or reject it based on your findings. Refer to the data and the graph, i.e. give me numbers!