*** Please do not write on this! Class Set!**

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(+/-).

$$\text{Average \% change} = \frac{\text{Absoulte value between average length before and after}}{\text{Larger average length value}} (100)$$

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!