

# The law of evidence



**ASSIGN  
BUSTER**

Smooth muscle is one of three muscle fiber types found in animals. Unlike skeletal and cardiac muscle cells, smooth muscle cells are not striated, and have single nuclei. Smooth muscles are typically under control of the autonomic nervous system, and do not contract voluntarily. Smooth muscle contracts slowly, and does not exhibit the characteristic twitch seen in skeletal muscle. In addition, smooth muscle is not prone to muscle fatigue, making it an ideal component of sphincter muscles. Smooth muscle is found in the gastrointestinal tract of many animals, and is responsible for peristaltic movements.

Smooth muscle contractions are affected by calcium and potassium ions. Calcium ion influx into the smooth muscle cell initiates a contraction. Potassium ion concentration in the extra cellular medium affects the resting membrane potential of the cell, bringing it closer to or farther away from its threshold voltage. Neurotransmitters affect different types of smooth muscle differently, depending on the association of the smooth muscle with excitable cells. In general, acetylcholine increases the muscle cell's permeability to calcium, while epinephrine decreases the cell's permeability to calcium.

## **Introduction and aim of the experiment**

The following report was to test a smooth muscle which was collected from the intestine of a rabbit. The main of this experiment was to see how the surrounding environment of the muscle could affect how muscle contracted. The experiment consisted of different environments and the muscle was monitored and results were recorded of the amplitude and the frequency of

the wavelengths. The levels of muscle contractions and relaxations were measured using a transducer, a D. C amplifier, and a laboratory computer.

### Overview of experiment

The smooth muscle was a small part of the intestine which was prepared correctly by trimming off the attached mesentery and fat. This experiment only required one piece of this smooth muscle and this was then placed into a beaker which was aerated and fed Ringer-Locke solution this is an aqueous solution containing the chlorides of sodium and potassium and calcium that is isotonic to animal tissues. The experiment only required one piece of small intestine, which had the length of 2-3 cm long. The intestine was held in place with a tissue holder, and was attached to the transducer via a piece of string. The transducer detected contractions and relaxation of the muscle, and via the D. C amplifier showed on the computer the frequency and strength of the contractions and relaxations. The solution that the muscle was placed in was kept at the temperature of 37 Celsius apart from when the environment condition changed was the temperature. This type of setup is known as 'in vivo' preparation. This preparation of the smooth intestine allows a precise control of the environmental conditions.

### **Brief description of test carried out**

The test which was carried out had six parts to it. The first part of the experiment was to gain initial control of the muscle this was done by having optimum conditions for the smooth muscle so it could achieve a steady rate of contraction and relaxation. This was achieved by adding Ringer-Locke solution and keeping it aerated. By having this set up it allowed the muscle

environment to be very closely met to the ideal body environment where the muscle would have a good contraction and relaxation rate. The muscle was left in this preparation until the readings on the computer were constant (about 4 minutes) the initial control was labelled using the comment bar.

The next environment was non-aerated this meant to turn the air supply off which was coming to the bath where the muscle was held. Then the Ringer-Locke solution was removed from the bath and fresh Ringer-Locke solution was then placed into the bath. The reason for removing the old solution was to prevent any inaccurate readings as the solution could still have contained oxygen which would have affected the results. This part of the test was recorded after every 5, 10, 15 minutes and the results were inserted into a table. The main purpose of using this environment was to see what the muscle contractions and relaxations are when there is a lack of oxygen. This part of the experiment was again labelled on the comment bar.

The next part of the experiment was to remove the Ringer-Locke solution and replace it with 50ml of glucose free solution and again results were recorded after every 5, 10, 15 minutes and recorded into a table.

The fourth different environment was change in temperature. The Ringer-Locke solution of 37 Celsius was replaced with a Ringer-Locke solution that was cooled to 4 Celsius. The purpose of this environment was to test the muscle activity in a cold environment and to analyse the effects.

The fifth environment involved the use of calcium free solution this replaced the Ringer-Locke solution. This was analysed for 5 minutes to see how the muscle activity was affected.

The final environment was to do with changes in the pH. The pH was changed from pH7 which is neutral to a different pH. The solution with different pH was prepared before hand and the purpose of this environment was to see what effect a pH change would have on the muscle activity.

After each part of the experiment initial control was established before moving on to the next part the reason for this being to keep the muscle running properly before each part of the experiment and to cause less damage to the smooth muscle. Also each part of the experiment was labelled on the comment bar this was done to show each different part clearly so it was not confused. (Clear methods are shown in the printouts)

### **Results table for my experiment**

Firstly the results achieved ere done by looking at the different graphs and to work out the amplitude for the graph the following was done:

### **Example (Graph not related to report)**

#### **To work out the amplitude of the graph recording two figures were recorded one being the peak of the wave and the other being the lowest part of the wave**

To work out the frequency for each part of the experiment the amount of waves were recorded in a minute time period. The frequency in this case was how many times the muscles contracted and relaxed in a minutes. The amplitude was the strength of each contraction and relaxing of the muscle. The maximum and minimum amplitudes were collected for each environment and recorded; the amplitude chosen was picked at random as

well as the minute where the frequency of waves was calculated. These are results are shown in the table below:

Environments

Frequency per Minute (min<sup>-1</sup>)

Amplitude 1

Amplitude 2

Maximum

Minimum

Maximum

Minimum

Initial control

15

2. 10

0. 78

1. 96

0. 65

Non aerated 5 Mins

13

1. 94

0. 47

1. 89

0. 38

Non aerated 10 Mins

17

1. 53

0. 36

1. 51

0. 38

Non aerated 15 Mins

16

1. 58

0. 38

1. 54

0. 36

Lack of glucose 5 mins

17

1. 34

0. 47

1. 49

0. 41

Lack of glucose 10 mins

17

1. 51

0. 43

1. 52

0. 45

Lack of glucose 15 mins

17

1. 37

0. 45

1. 43

0. 46



Cold ringer solution

5 Mins

14

1. 76

0. 44

1. 73

0. 51

Calcium Lack 5 Mins

12

1. 15

0. 63

. 95

0. 47

Change of pH

Flat – No waves

—  
—  
—  
—

## **Discussion of results**

Firstly a diagram of the intestine is needed to show how it works and what different types of cells it contains. This is needed as it will help to understand why the muscle behaved differently when tested with six different environments. A diagram of the small intestine is shown below;

The small intestine contains the 4 basic layers which are serosa, muscularis, submucosa, and mucosa.

Small intestine wall is composed of the same four layers that make up most of the gastrointestinal tract: serosa, muscularis, submucosa, and mucosa.

The mucosa is composed of a layer of epithelium, lamina propria, and muscularis mucosae. The epithelial layer of the small intestinal musoca consists of simple columnar epithelium that contains many types of cells.

Some of these are the following:

Enteroocytes – these help with the transport of substances from lumen of the intestine to the circulatory system, synthesis of the glycoprotein enzymes needed for terminal digestion and absorption.

Goblet cells – these are unicellular mucin also known as secreting glands.

Paneth cells – these are located at the bottom of the intestinal glands. Their main function is their secretion of granules which contain lysozyme this

<https://assignbuster.com/the-law-of-evidence/>

enzyme helps breakdown bacteria also known as phagocytosis. Paneth cells may have a role in regulating the microbial population in the small intestine.

Enteroendocrine cells – these are mostly found again in the lower parts of the intestinal gland known as the crypt. The main function of these cells is to release several hormones. The main one being cholecystokinin, secretin and gastric inhibitory peptide these help increase pancreatic and gallbladder activity.

Intermediate cells – these are young enterocytes and goblet cell which are able to withstand cell division.

Apart from the smooth muscle many other cells and vessels make up the intestine. The small intestine also contains submucosal artery and vein, lymphatic vessel, submucosal plexus, circular layer of smooth muscle, and myenteric plexus. All these tissues, cells, and vessel combine to make the small intestine wall.

The smooth muscle in the control environment was able to obtain a steady frequency through out its 4 minute period with 15 waves per minute. The waves which were seen were the smooth muscle contracting and relaxing. The amplitude levels of the waves were both quite high showing strong contractions rate the reason for this was that the environment set was to ideal conditions where the muscle could perform its best. It had a max amplitude of 2.10 and min amplitude of 0.78 which shows that having ideal conditions the muscle is able to behave normally without any problems.

The effect of oxygen lack of the smooth muscle cause the small intestine to increase the frequency, at 5 minutes no aerated the frequency had first dropped to 13 and after 15 minutes the frequency of contraction and relaxations had increased to 17 the reason or this being without oxygen the smooth muscles started to have spasms as it was unable to contract properly without the oxygen supply needed. Also the amplitude levels decreased quite quickly from 1.94 (max) and 0.47(min) at 5 minutes to 1.58(max) and 0.38 (min) at 15 minutes. The reason for this was that without oxygen the muscle was unable to make the energy needed for strong contractions as the peak is lowered as can be seen on the traces. This was detected by the pull on the string that was attached to the transducer; the pull was not as strong so this was recorded on the traces. So without oxygen the muscle cells are still able to make ATP but a small amount. Only about 2 ATP are produced per molecule of glucose in glycolysis. If there is no oxygen present, the Pyruvate produced in glycolysis undergoes fermentation to regenerate the NAD<sup>+</sup> used in glycolysis. This is known as anaerobic respiration, anaerobic respiration generates only two ATPs, and lactic acid is produced. Most lactic acid diffuses out of the cell and into the bloodstream and is subsequently absorbed by the liver. Some of the lactic acid remains in the muscle fibers, where it contributes to muscle fatigue. Because both the liver and muscle fibers must convert the lactic acid back to pyruvic acid when oxygen becomes available, anaerobic respiration is said to produce oxygen debt

The next part of the experiment was to test how the muscle activity differed when placed in glucose free solution. From this part of the test the frequency

of muscle activity stayed consistent throughout the 15 minutes. The traces show consistent movement and also the amplitudes levels differed as at 5 minutes (1. 34) the maximum amplitude was low then at 10 minutes (1. 51) it was higher and at 15 minutes (1. 37) it decreased again to a similar figure which was at 5 minutes. By looking at this result the results are not as accurate as they should have been, meaning they may have been some kind of inaccuracy when following the method as without glucose, ATP can not be made and the amplitude of the waves should have been lower.

ATP can be made from glucose which is stored in the carbohydrate glycogen. Through the metabolic process of glycogenolysis, glycogen is broken down to release glucose. ATP is then generated from glucose by cellular respiration. Also ATP can be produced from glucose and fatty acids obtained from the bloodstream. When energy requirements are high, glucose from glycogen stored in the liver and fatty acids from fat stored in adipose cells and the liver are released into the bloodstream. Glucose and fatty acids are then absorbed from the bloodstream by muscle cells. ATP is then generated from these energy-rich molecules by cellular respiration. Without glucose the frequency should have increased but the amplitudes levels should have decreased as there was not a sufficient energy source which could supply the muscle so it could contract and relax.

The next part of the experiment consisted of placing the smooth muscle into a cooled solution of 4 Celsius from a change of 37 Celsius. There was not much change to the frequency but it did drop a little bit due to the muscle not being used to these environmental conditions. The amplitude differed from the control readings as they had decreased but were still quite high as

they had the nutrients in the solutions which helped them to contract. If this experiment was left to carry on then there would be further change as the solution would gradually heat up to room temperature and this would mean that the smooth muscle activity would increase.

Calcium plays a big part in all muscle contraction as well as smooth muscle contraction which is different as it does not contain troponin. In smooth muscles calcium ions enter from outside the cell. They then bind to an enzyme complex on myosin; this then breaks up ATP into ADP and then transfers the Pi directly into myosin. By doing this it allows the myosin to activate and form cross bridges with actin. When the calcium is pumped out of the cell, the Pi gets removed from myosin by an enzyme this allows the myosin to become inactive and the smooth muscle is able to relax. This process is also known as myosin regulated contraction.

In the experiment where calcium free solution was added it affected the smooth muscle immensely as the frequency of contracting and relaxing dropped to 12. Also the amplitude levels came down as the contractions and relaxations levels were not strong the max being 0.95 and the minimum being 0.47 if this was left for a longer period of time the frequency levels may have dropped more. Without calcium entering the cell the smooth muscle is unable to do the process which is described above. While making the Ringer-Locke solution it is not only the calcium ions which are important to the smooth muscles. Some others are potassium chloride and sodium chloride, the reason these ions are needed because it helps to portray an environment such as the body with ideal conditions. If only distilled water was used it would mean the cells in the muscles would up take the water and

blow up. So these ions are used so they are able to keep a concentration gradient and allow everything to work correctly as it would in the smooth muscles natural environment.

The final environment was the change in pH levels. The results showed that the muscle had stopped functioning and there was no reading on the traces. This meant that there was no muscle contraction or relaxing. The reason for this was the muscle had broken down the reason for this muscle fatigue was that the low pH had affected the smooth muscle as it was unable to perform in this type of environment. Also as the low pH solution was there for a certain period of time the muscle was unable to remove it and therefore caused the muscle to breakdown. The low pH may have affected the sarcoplasmic reticulum which may lead to the interfere of the intercellular calcium concentrations, this can lead to long term physical muscle damage as muscle fibers are affected.

While preparing the isolated smooth muscle many precautions are taken so the muscle can avoid excessive pH changes. One of these precautions which are taken is to make sure that before inserting the small intestine into the bath. The solution will need to be tested with pH indicator test strips which will give a fairly accurate reading of the solution which the smooth muscle will be placed. This is very important as if the pH is incorrect it will mean that the smooth muscle will not perform to its full potential meaning the results achieved will be inaccurate. To gain accurate results all solutions which are used will need to be checked to see if they are the correct pH by using the pH indicating strips. After this test the smooth muscle was unable to reach the control again as the muscle had broken down.

If this experiment was done again at room temperature the results would differ as the performance of the muscle would decrease. This is because the optimum temperature inside the body is around 37 Celsius and this temperature allows the muscle to work at an optimum rate. The lower temperature will mean that muscle contraction will be slower as there will be an effect on enzyme reactions as the more heat there is the more kinetic energy there is this will mean that the muscle activity will be good.

If acetylcholine was added to the solution bath of the smooth muscle the membrane potential would decrease and the frequency of waves would increase. The muscle will become more active, with an increase in tonic tension and the number of rhythmic contractions. The effect is mediated by enzymes which increases the intracellular Calcium concentration. Another substance which could have been added was adrenaline. Adrenaline allows blood to flow more easily to your muscles. This means that more oxygen is carried to your muscles by the extra blood, which allows your muscles to function at elevated levels. Adrenaline also facilitates the conversion of the body's fuel source (glycogen) into its fuel (glucose). This carbohydrate gives energy to muscles, and a sudden burst of glucose also allows muscles to strengthen further.

## **Skeletal and smooth muscle muscles differences in structure and function**

There are many differences between the two types of muscles the differences are stated below:



## **Smooth muscles**

### **Skeletal muscle**

A smooth muscle fiber has no T tubules, and the sarcoplasmic reticulum forms a loose network throughout the sarcoplasm.

Are long cylindrical cells that contain many nuclei

Smooth muscle tissue has no myofibrils or sarcomeres

They are striated this shows their precise alignments of their myofilaments.

This tissue also has no striations and is called nonstriated muscle.

Thick filaments consist of myosin as thin filaments consist mostly of actin.

Thick filaments are scattered throughout the sarcoplasm of a smooth muscle cell

Each independent cell is stimulated by a motor neurone.

Adjacent smooth muscle cells are bound together at dense bodies, transmitting the contractile forces from cell to cell throughout the tissue.

Connective endomysium separates cell

### **Function Differences**

Smooth muscles cells are an involuntary action and can work slower so they do not have muscle fatigue.

Skeletal Muscle contains both Fast & Slow Twitch muscle fibers, that allow for a faster reaction where needed, and the opposite is true for the Slow as well

Smooth muscle lines your arteries and airways and serves to contract or relax to help control blood pressure.

skeletal muscles function almost continuously to maintain your posture making one tiny adjustment after another to keep your body upright

They are also present in the iris of the eye to control the size of the pupil in response to light. By the use of the radial and circular muscle.

Skeletal muscle is also important for holding your bones in the correct position and prevents your joints from dislocating. Some skeletal muscles in your face are directly attached to your skin

They line the GI tract to move “ food” through the intestines. This is done by peristalsis.

Skeletal muscle generates heat as a by-product of muscle activity. This heat is vital for maintaining your normal body temperature.