STUDENT'S NOTES

Properties of Skeletal Muscle

The Preparation

Isolation and removal of the preparation from the frog is relatively straightforward and is summarised below:

1. The frog is stunned and pithed, a procedure which destroys the brain and spinal cord.
2. The skin covering the lower half of the body is removed.
3. The urostyle is cut free and removed exposing the roots of the sciatic nerve on both sides. A ligature is tied around the nerve and the nerve is cut central to the ligature.
4. The nerve is carefully dissected free of surrounding connective tissue down to the knee joint. Its path through the thigh may be more easily seen if the muscles are gently parted using the thumbnails.
5. Care must be taken not to touch the nerve with dissecting instruments or to stretch it unduly.
6. The in-situ length of the gastrocnemius muscle is measured and a ligature tied around the achilles tendon. This should be left long to facilitate attachment to the strain gauge.
7. The tendon is cut and the gastrocnemius muscle carefully dissected free up to the knee joint. The limb bones either side of the knee-joint are cut with coarse scissors allowing the muscle, nerve and knee joint to be lifted free of the animal.

The organ bath

The organ bath consists of a perspex lidded box containing a series of metal electrodes spaced 5 mm apart. The nerve and muscle are laid across the electrodes. It is important to keep the preparation moist with frog Ringer solution throughout the experiment.

The muscle length is fixed at an optimal length (slightly stretched) by pinning at the knee and attaching the ligature tied around the achilles tendon to the strain gauge. The pin should be inserted through the coarse connective tissue covering the patella to ensure that the nerve is not damaged. The ligature thread used should be pre-stretched.

Stimulation and recording

The nerve is stimulated electrically from an isolated stimulator. The voltage, stimulus duration and stimulus frequency may be controlled and there is a facility to deliver either single or paired stimuli. It is also possible to directly stimulate the muscle. For most experiments the stimulus voltage will be in the range 0-10V, the duration will be 50μs and the frequency of stimulation 0.1 Hz. Isolated muscles fatigue rapidly if stimulated at high frequencies for extended periods.

Records of compound nerve action potentials and muscle action potentials are obtained form the recording electrodes on the nerve and muscle respectively. The records are amplified (AC amplifier) and displayed on an oscilloscope.

Contractile tension is measured isometrically using a strain gauge transducer which converts a tension change into a voltage. The strain gauge output is amplified (DC amplifier) and displayed on the second channel of an oscilloscope. By calibrating the strain gauge it is a simple matter to convert the displayed voltage to a tension change and express the tension in g. Note that skeletal muscle contractions can also be measured isotonically where changes in muscle length are measured using an isotonic transducer.

Q1 Draw a diagram of the preparation as it is setup in the organ bath to allow the nerve to be stimulated electrically and the following to be recorded: (i) the compound nerve action potential (CNAP); (ii) the muscle action potential; (iii) the isometric force of contraction (strain gauge). Indicate the positions of the recording and stimulating electrodes.

Q2 Explain briefly how you would calibrate the strain gauge to enable you to convert the voltage change to tension (g).

Experiment A - Stimulus strength-response relationship.

Aim: to demonstrate the relationship between the stimulus voltage applied to the sciatic nerve and the muscle tension.

Task: measure the peak muscle tension produced by each stimulus voltage from the monitor screen and complete the table below.

Stimulus voltage (V)	*Muscle Tension (g)*
0.6	
0.8	
1.0	
1.4	
1.6	
1.8	
2.0	
2.6	
3.0	
4.0	

On graph paper draw a graph of muscle tension in g (y-axis) versus stimulus voltage in V (x-axis).

Q3 Explain the relationship between the stimulus voltage applied to the nerve and the muscle tension produced.

Experiment B - Effect of second stimulus

Aim: to investigate summation

Task: the sciatic nerve is stimulated with paired supramaximal stimuli such that the second stimulus is delivered at different times during the contraction evoked by the first stimulus. The intervals have been set such that the second stimulus is delivered (i) during the relaxation phase - 130ms (ii) at the peak of the contraction - 70ms (iii) during the contraction (25ms).

Draw the responses for (show the single contraction in diagram (iii))

(i) (ii) (iii)

Q4 The muscle tension resulting from the second of the two stimuli is greater than that from the first in all three situations - this is summation. Try to explain these results bearing in mind that the stimulus voltage applied to the nerve is supramaximal (i.e. it will activate all nerve fibres).

Q5 In this experiment the minimum stimulus interval used was 50ms. Would the refractory period of the nerve have any relevance in this experiment?

Experiment C - Tetanus

Aim: to investigate the effects on the muscle response of stimulating the nerve at high frequency.

Task: stimulate the nerve using a supramaximal voltage either to produce an **unfused tetanus** (start at 1Hz, increase to 10 Hz for 4s and then return to 1Hz) or a **fused tetanus** (start at 1Hz, then 10 Hz (1s), then gradually over 3s increase to 70 Hz, back to 10 Hz then 1 Hz.

Measure the peak/plateau tension produced during the unfused and the fused tetanus and complete the table below.

Stimulation frequency (Hz)	*Muscle Tension (g)*
10	
70	

Q6 Explain why the tetanic tension developed by the muscle is significantly greater than that developed during a twitch contraction.

Q7 Which of the following most closely resembles the way in which a muscle contracts in the body: a twitch contraction or a tetanic contraction? Explain your answer.

Experiment D - Length-tension relationship.

Aim: to investigate the effect of changing muscle length on active and passive tension.

Task: Measure the isometric tension (either in mm or convert to g) of sartorius muscle developed at different muscle lengths: (i) during passive stretching of the muscle in 1mm increments from its flaccid length - PASSIVE TENSION: (ii) when the muscle is stimulated tetanically at each length (2s; supramaximal; 50Hz) - ACTIVE (contractile) TENSION.

Note the equilibrium (flaccid) length of the muscle is 26mm

Muscle Length (mm)	*Passive Tension*	*Active Tension*
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		

Q8 Explain the terms active tension and passive tension.

Q9 Explain the observed results with reference to the sliding filament theory of muscle contraction.

Q10 From your results determine the resting length of the muscle.

Experiment E - Effect of curare.

Aim: to investigate the action of curare on the compound nerve action potential (CNAP) and the isometric muscle tension in the sciatic nerve-gastrocnemius muscle preparation.

Task: stimulate the nerve supramaximally at 1 Hz for the duration of the experiment. Observe and measure the CNAP and the isometric muscle tension before and at 50s and 100s after the dropwise application of curare to both the nerve and the muscle. Complete the table.

Time (s) after curare	*amplitude of CNAP (mm)*	*Muscle Tension*
Before curare		
50		
100		

Q11 Explain the action of curare.

Q12 What was the effect of stimulating the muscle directly when the muscle response evoked by stimulation of the nerve had disappeared?