Pharmacology – Working with isolated tissue preparations

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This material is derived from the work of A/Prof Elizabeth Davis, Monash University, and pertains to the procedures used by the Dept of Pharmacology at Monash University.

The isolated tissue setup

In order to keep a tissue viable outside the body (ie an isolated tissue preparation), certain requirements must be met. For example, the tissue must:

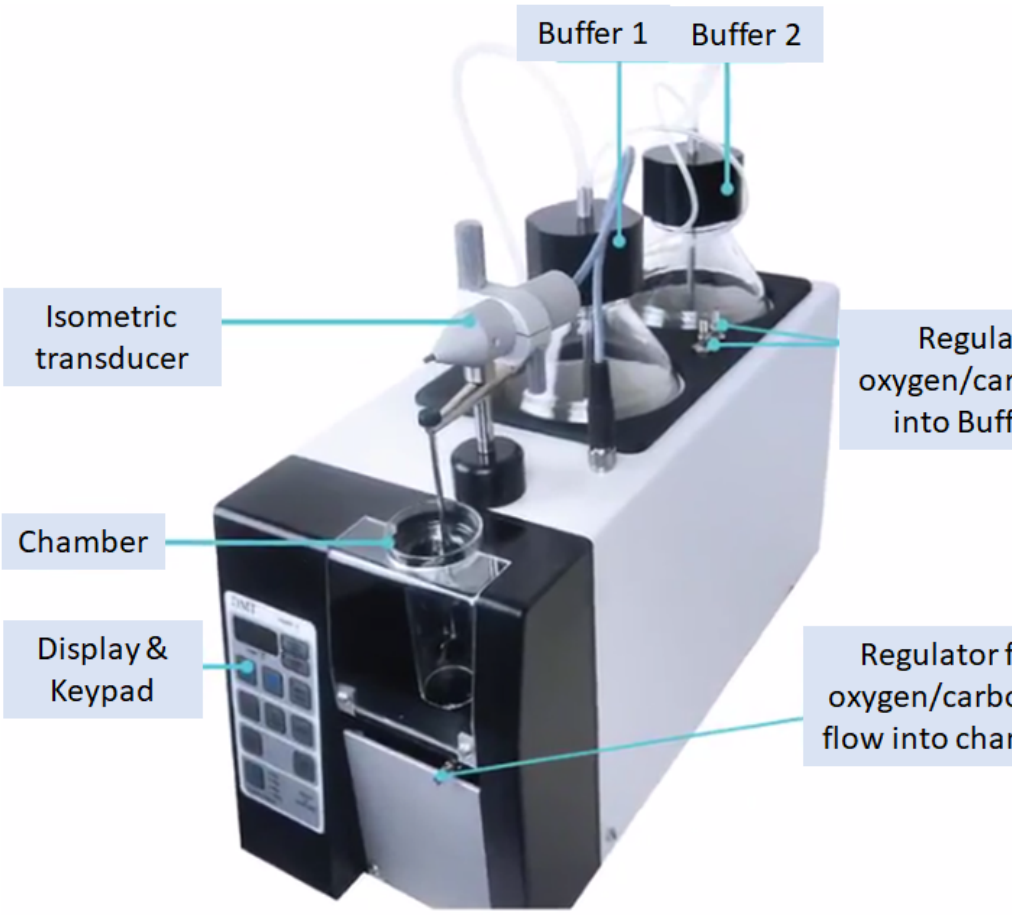
- be maintained at a constant temperature (usually approximately body temperature)
- receive a constant (and adequate) supply of oxygen
- be provided with a source of energy (e.g. glucose)
- be provided with the necessary ions for cellular function (e.g. Na^{+}, Cl^{-}, Mg^{++}, Ca^{++})

The minimum requirements for an isolated tissue experiment, therefore, are

- **a heated organ bath**,
- **an appropriate physiological salt solution** (PSS) and
- **an oxygen supply** (usually carbogen – 95%O_2/5%CO_2).

The reason for using 95% O2 rather than atmospheric levels of 21% O2 is to compensate for the lack of hemoglobin or other oxygen carriers (usually in the blood to the tissue) in the artificial perfusate solutions in the organ bath. In this way, we can ensure adequate delivery of oxygen to the tissue.

In the photo below, the chamber at the front (beside the keypad) contains the glass organ bath which is shown in a close-up photo under the label "Setting up procedure".



If the aim of an experiment is to examine muscle (smooth, cardiac or skeletal) responses, the muscle will need to be connected to a recording apparatus to allow recording of its force (also called muscle "tension"), developed as it contracts.

In the experiments you will be undertaking, the tissues will be connected to a PowerLab recording apparatus via an **isometric force transducer**.

When measuring changes in muscle tension in response to drugs, it is usual to put the muscle

under a basal resting tension, so that you can measure changes from a resting level.

Physiological Salt Solutions

The physiological fluid used in most experiments in pharmacology practicals at Monash University is Holman's solution which contains:

	mmol/l	g/l
NaCl	120	7.0
KCl	5	0.372
$NaHCO_2$	25	2.1
NaH_2PO_4	1	0.156
$MgSO_4$	1	0.246
Glucose	11	1.981
Sucrose	10	3.423
$CaCl_2$	2.5	0.367

All the salts except calcium chloride can be dissolved together.

Concentrated solutions may be used if preferred but sodium bicarbonate will not keep and the solid salt should be weighed out fresh every day.

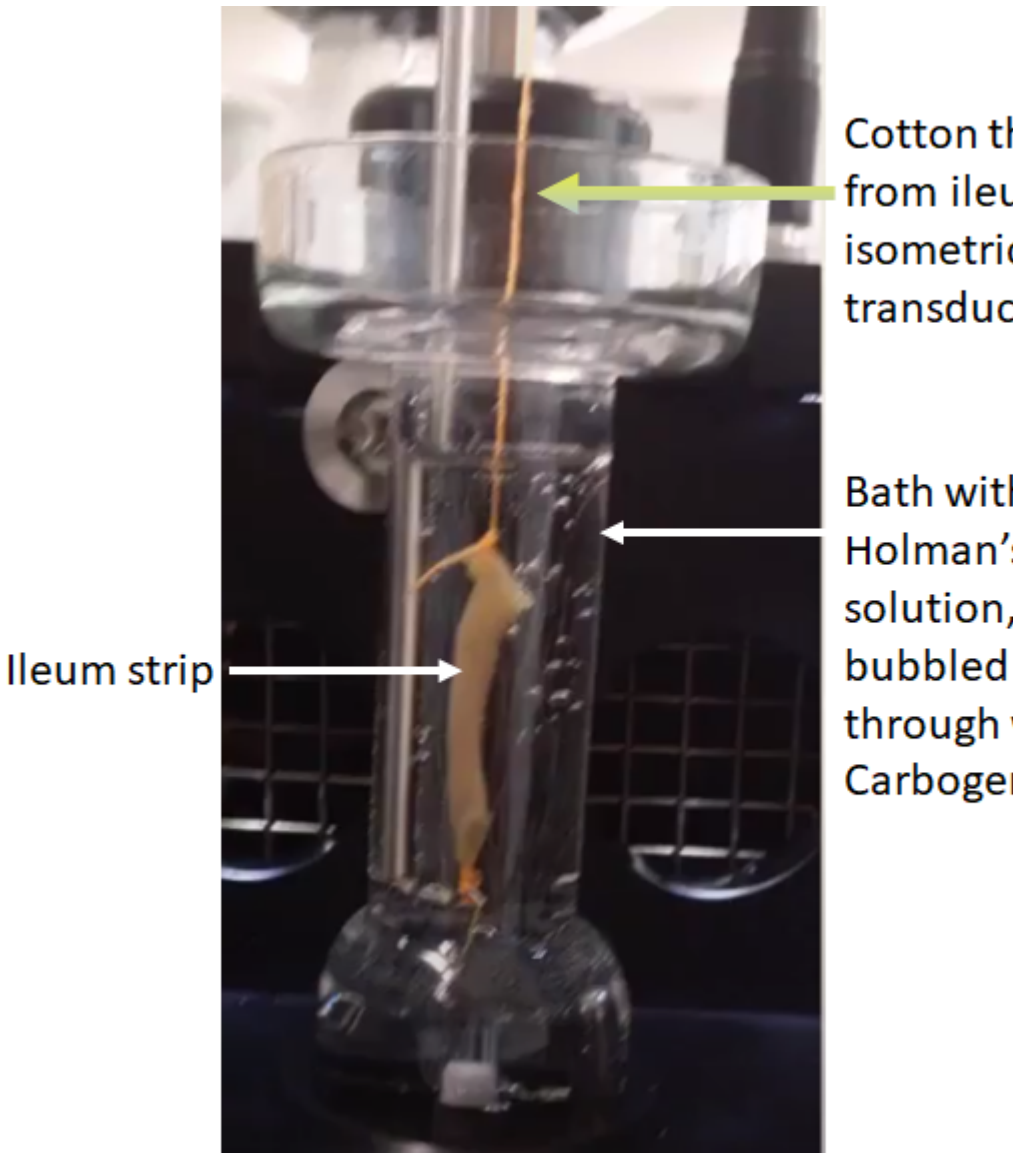
When all the salts have been dissolved in the majority of the final volume, the calcium chloride should be dissolved in the residual volume and bubbled with carbogen, then added to the rest with stirring.

The final volume should be adjusted and then the fluid gassed with carbogen for 15 min before use.

The pH of this solution should be 7.4.

Setting up procedure

In most pharmacology practical class learning sessions, isolated organ preparations will be set up for you and the recording apparatus (PowerLab / Chart) will be calibrated. However, the following notes are intended to provide a reference for the general procedures involved in the setting up of isolated tissue experiments.



All the physical aspects of the experiment should be organized before the biological tissue is obtained. These checks should be made before you start to dissect and set up the preparation.

1. **Calibrate the PowerLab and the force transducer** so that you can actually measure

the responses of the tissue in some absolute unit(s).

2. Check and mark the volume of the organ bath. **This must be known so that you can calculate the final bath concentrations of the drugs you add to the chamber**.
3. Obtain the correct physiological salt solution (PSS) for your experiment. **Stock solutions should have been bubbled with carbogen ($95\%O_2/5\%CO_2$) in the main reservoir** and should appear quite clear.
4. **Check the temperature of the outer bath**.
5. **Fill the tissue bath with PSS and ensure that it is being bubbled adequately with the correct gas mixture** (usually carbogen). Make sure that any distilled water remaining in the glass coils has been flushed through and that your tissue bath does contain PSS.
6. **Check that you have separate pipette tips for each drug** (and, if necessary, each of its dilutions) you will be using.

Tips for working with isolated tissues

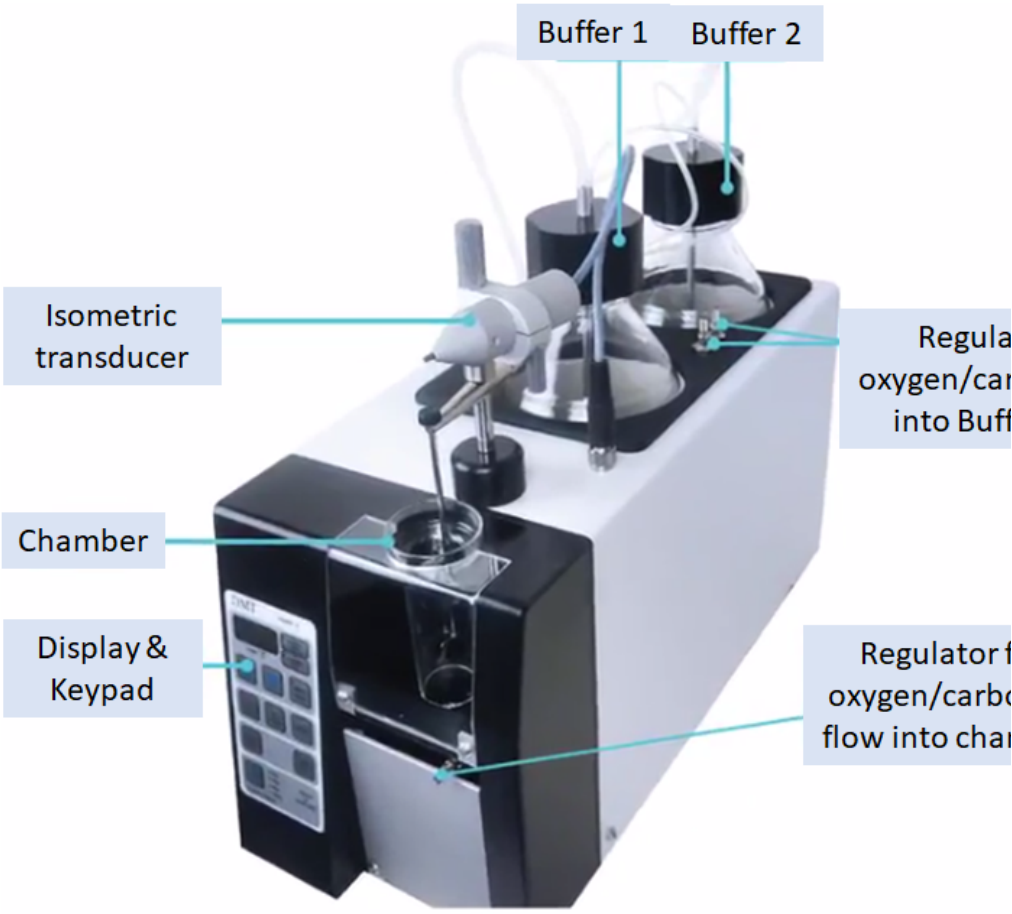
The following points will help you to carry out the experiments successfully.

1. The volume of the organ baths in the Monash Dept of Pharmacology practical class is 20 ml. This must be known so that calculations of final bath concentrations can be made.
2. Check that the isolated tissue is continually bathed in the appropriate physiological salt solution (PSS). Try to keep the reservoir quarter- to half-full throughout the experiment. **YOU WILL NEED TO CHECK THIS REGULARLY.**
3. For most preparations, drugs will be washed from the organ bath using the overflow method. You will be shown how to do this using short bursts of fluids. It is wise to practice this a few times before commencing drug additions.
4. Ensure that the preparation is being bubbled adequately. This is necessary to provide adequate mixing of the drugs added to the bath as well as to provide oxygen to the tissue and keep the solution correctly buffered.

5. Check that the tissue, bath, and transducer are aligned vertically to maximize the measurement of changes in tissue tension. **Check this alignment periodically throughout the experiment** as sometimes (eg when the tissue tension decreases) the cotton attaching the tissue to the transducer becomes stuck on the side of the organ bath and it may appear that the tissue is no longer responding to drug additions.
6. Use separate pipette tips for each drug (and each dilution) you will be using. You *can* use the same pipette tip with **increasing** concentrations of a drug (e.g. when doing a concentration-response curve), but if a more dilute solution is to be used (e.g. starting a new c-r curve), you will need to use a new tip.
7. Before adding drugs to the tissue bath, check with your partner (and/or a demonstrator) that you have calculated the volume and the dilution factor correctly.
8. Take time to familiarise yourself with the correct use of the pipette – especially the two stop points and the tip ejector button.

Experimental procedures

Obviously, detailed procedures can vary considerably from experiment to experiment, according to particular aims, to the special properties of the tissues, to the drugs used and to the desired form of statistical handling of results. Therefore only the more important and widely applicable guidelines can be set out here.



Noting down information (*yes, you do forget later!*)

Every experimental record (including PowerLab Chart files) should bear the following information:- date, tissue, conditions (e.g. bathing solution and temperature; resting tension), operator's name(s).

This information should be included in the NOTEPAD of the Chart file containing your data.

Equilibration period

Before adding any drugs or stimulating nerves, allow the tissue to reach a steady baseline.

This **equilibration period** should be at least 15 min, during which time the tissue should be washed (by overflow) at least once.

For most tissues, it is advantageous to extend this equilibration period to 30 or 40 min, so long as the tissue is washed occasionally.

Activating the tissue electrically through the nerves

When using innervated preparations (i.e., tissues with their nerve supply that controls or modulates their activity), carefully check the particular stimulus parameters to be used for that experiment.

If the tissue fails to respond to nerve stimulation, check that the electrode terminals are connected to the stimulator and that this is switched on.

If all else fails, call a staff member!

How to add drugs to the organ bath

When adding drugs to the bath, use an automatic pipette and removable tip and inject the drug well into the bath, avoiding a direct 'hit' on the tissue. Be sure that the tip is firmly attached to the pipette so that it doesn't drop off into the bath.

Allow agonist drugs to remain in the bath only until the tissue just starts to plateau from the drug effects (i.e. just after the peak of the contraction – see below) or for a set time period if appropriate (as instructed in manual).

Once this contact period is determined for a tissue it should be kept constant for the experiment.

To remove agonist, wash with at least twice the bath volume. The tissue should then return to its original baseline.

Occasionally '**washout responses**' will be seen (usually as a contraction during or just after the wash). This may be avoided by using several short

bursts of washing rather than a long continuous overflow.

Comparing drugs

When comparing the relative potencies of two drugs or when assaying a solution of unknown strength, it is a good idea to arrange your dilutions so that similar volumes of both solutions are added to the bath.

The volume of drug added to a 20ml bath is best kept to between 20 and 200 microlitres

Try to avoid adding large volumes (eg >500 microlitres) as large volumes dissolved in a medium other than physiological saline can alter the ionic balance and perhaps the temperature of the bathing medium – which in themselves may cause a change in muscle tension.

It is also a good idea to occasionally check the effect of an injection of drug '**vehicle**', using a volume equal to the maximal volume of the drug you will inject.