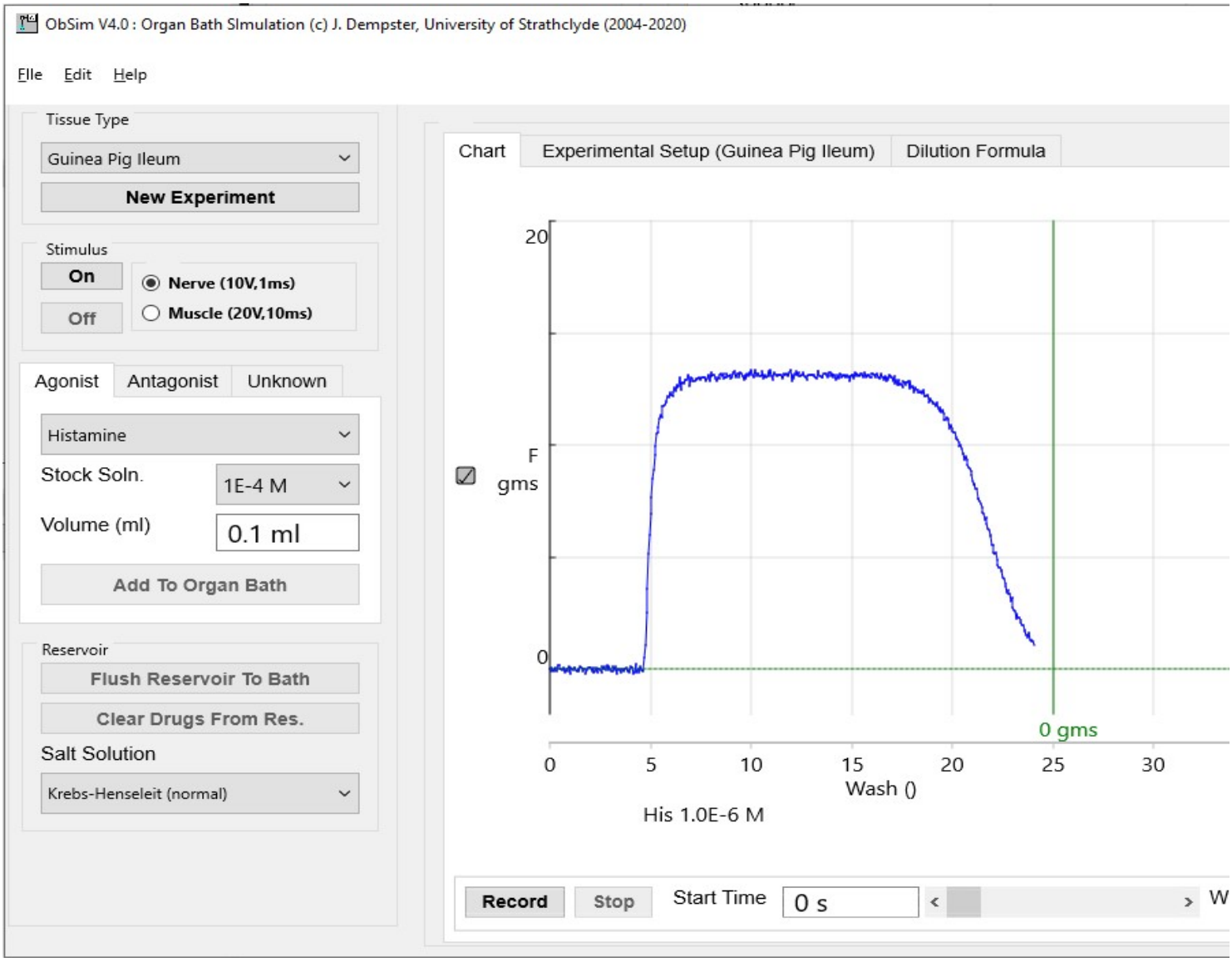
OBSIM - Organ Bath Simulation

Table of Contents

Introduction

The OBSIM (**O**rgan **B**ath **SIM**ulation) program simulates a classical, i*n vitro,* pharmacological experiment using one of five different types of tissue: guinea pig ileum, rabbit jejunum, chick biventer cervicis, rat artery and rat phrenic nerve-hemidiaphragm.



In vitro, pharmacological experiments on isolated organs or tissues provide a means of discovering or quantifying the effects of drugs on specific tissues before their application in humans or in living animals. The properties of most drugs currently in use were elucidated using this method and, *in vitro*, experiments continue to be an essential stage in the drug discovery process.

Tissues Types

Five types of tissue are available for study:

Guinea pig ileum: A section cut from the ileum region of a guinea pig's gastrointestinal tract. The smooth muscle within the ileum contracts in response to the application of a variety of agonists. The enteric nervous system within within it can also be electrically stimulated to produce contraction.

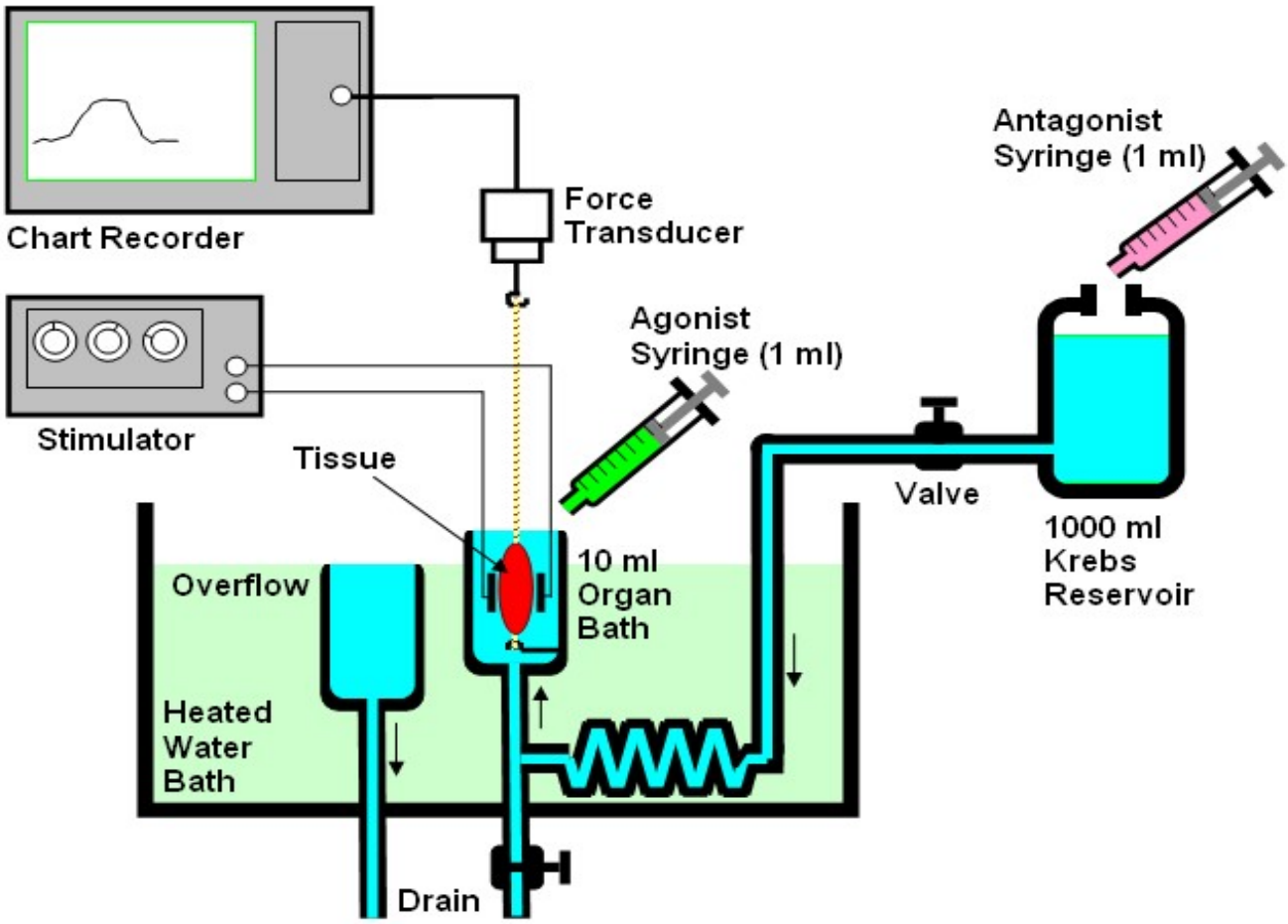
Chick biventer cervicis: A skeletal muscle from the neck of a young chicken, dissected with its innervating nerve intact. It can be made to contract by the application of agonists or by electrical stimulation of the nerve.

Rabbit arterial ring: A section cut from a rabbit artery and attached to a force transducer. It can be made to contract by the application of either KCL or the agonist noradrenaline. The artery can be made to relax again by applying a number of antagonists.

Rabbit jejunum (Finkleman's preparation): A section cut from the jejunum region of a rabbit's gastrointestinal tract, along with the associated sympathetic nervous innervation. It contracts spontaneously and these contractions can be inhibited by the application of adrenergic agonists or by stimulation of the sympathetic nerves.

Rat phrenic nerve-hemidiagphragm: A skeletal muscle from a rat dissected with its associated nerve. It can be made to contract by the application of agonists or by electrical stimulation of the nerve.

Apparatus



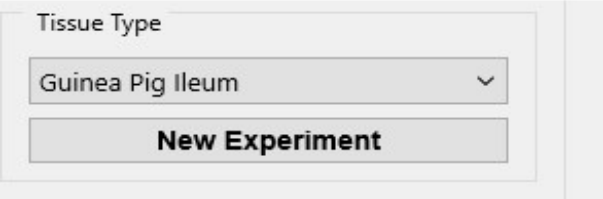
The tissue under study is immersed in a **10 ml** volume organ bath containing a physiological salt solution, Krebs-Henseleit (K-H), which approximates the extracellular fluids normally bathing the tissue *in vivo*. The organ bath is contained within a Perspex bath that contains water maintained at a temperature close to the normal body temperature of the animal (37°C) by a heater and thermostat.

Drugs can be applied to the tissue by pipetting small volumes of drug-containing solution directly into the the organ bath and removed by flushing the organ bath with fresh solution from a reservoir containing K-H solution. Opening the reservoir tap allows physiological solution to flow through the warming coil into the organ bath. A mixture of oxygen (95%) and carbon dioxide (5%) is bubbled into both the reservoir and organ bath to provide oxygen and maintain the pH of the tissue. The tissue can also be stimulated electrically using a stimulator attached to a pair of electrodes placed on either side of the tissue within the organ bath.

The tissue is attached to a force transducer which generates an electrical signal proportional to the contractile force generated by the tissue when a drug is applied. This is connected to an amplifier that boosts the small voltages produced by the transducer to a level suitable for measurement by the computer. The amplified signal is then fed into an analogue-to-digital (A/D) converter which digitises the signal and stores it in the computer, under the control of a digital chart recording program.

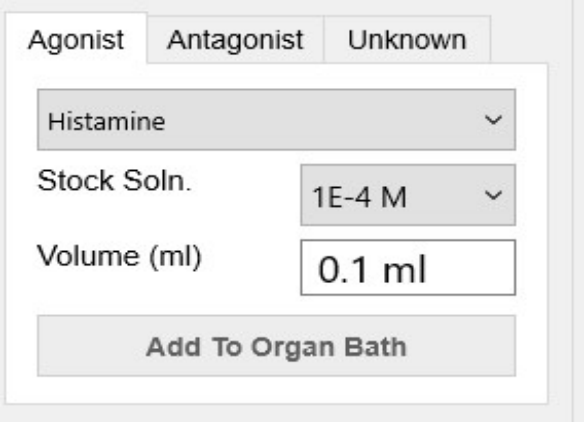
Starting an Experiment

a) To start a new experiment, select the type of tissue to be placed within the organ bath from the Tissue Type and click the **New Experiment** button.



b) Click the **Record** button to start the chart recorder running.

c) Select and apply drugs from the list of available Agonist, Antagonist or Unknown drugs.

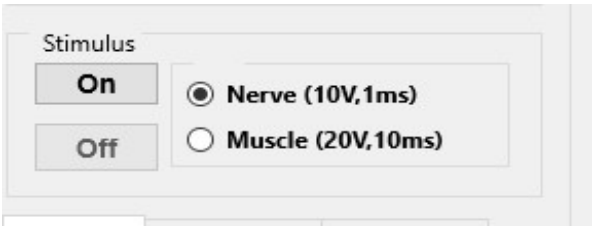


Electrical Stimulation

Guinea Pig Ileum, Rat Diaphragm, Chick Biventer

To electrically stimulate the tissue, select **Nerve** to stimulate the nerve fibres innervating the muscle tissue or **Muscle** to directly stimulate the muscle fibres within the tissue.

Then click the **On** button to start stimulating at regular intervals.



Click the **Off** button to stop stimulating.

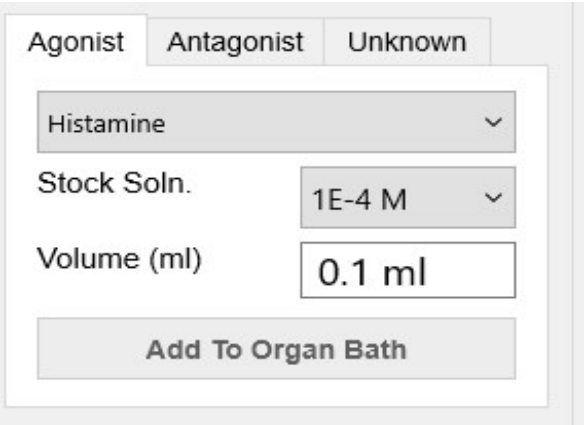
Rabbit Jejunum

To electrically stimulate the sympathetic nerve supply to the jejunum, set the stimulus frequency in the **Frequency** box and click the **On** button to start stimulating at regular intervals.

Adding Agonists

To add an agonist drug to the organ bath:

a) Select the **Agonists** page.



b) Select the type of agonist to be applied from the list of available agonists.

c) Select the concentration of the drug solution to be applied from the **Stock Soln.** list.

d) Enter the volume (between 0 and 1 ml) of the stock solution to be applied into the **Volume** box.

e) Click the **Add to Organ Bath** button to add the selected volume of the select stock solution agonist into the organ bath.

f) When the tissue response on the chart recording reaches a steady state (or if no response has occurred after 30 seconds) click the **Flush Reservoir to Bath** button to wash out the agonist from the organ bath.

Notes.

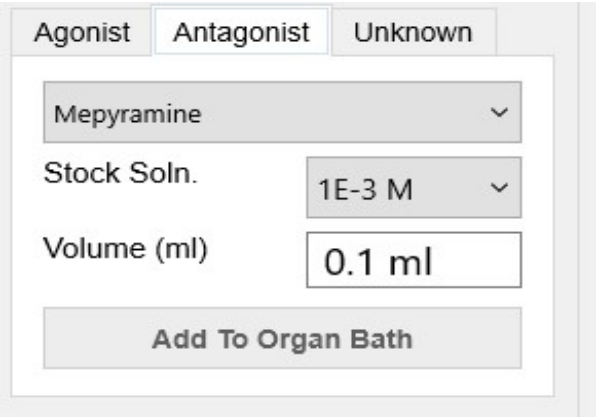
Agonists are drugs which, when applied to tissue, cause a response (contraction in the case of the tissues typically studied in organ baths) by binding to specific receptors on the surface of the cells within the tissue.

Agonist	**Receptors**
Histamine	Histamine receptors
Carbachol	Cholinoceptors
Morphine	Mu-opioid receptors
Loperamide	Mu-opioid receptors
Phenylephrine	$Alpha_1$-adrenoceptors
Acetylcholine	Cholinoceptors
Pilocarpine	Muscarinic cholinoceptors

Adding Antagonists

To add an antagonist drug to the organ bath or Krebs' solution reservoir:

a) Select the **Antagonists** page.



b) Select the type of antagonist to be applied from the list of available antagonists.

c) Select the concentration of the drug solution to be applied from the **Stock Soln.** list.

d) Enter the volume (between 0 and 1 ml) of the stock solution to be applied into the **Volume** box.

e) Select **Organ Bath** from the **Add To** list if the antagonist is to be applied directly to the organ bath. Select **Reservoir** if the drug is to be added to the solution reservoir.

f) Click the **Add to** button to inject the volume of the selected stock solution agonist into the organ bath or reservoir.

Notes.

Antagonists are drugs which block the actions of agonists on tissue, reducing or preventing the tissue response. Application of an antagonist will thus have no apparent effect on the tissue in the organ bath unless an agonist is present or the tissue is being nerve stimulated.

Antagonists are typically studied by observing the effects an antagonist has on different concentrations of a chosen agonist. To avoid the necessity of repeatedly applying the antagonist to the organ bath before each agonist, it is usually added to the solution reservoir used to flush the organ bath between agonist applications.

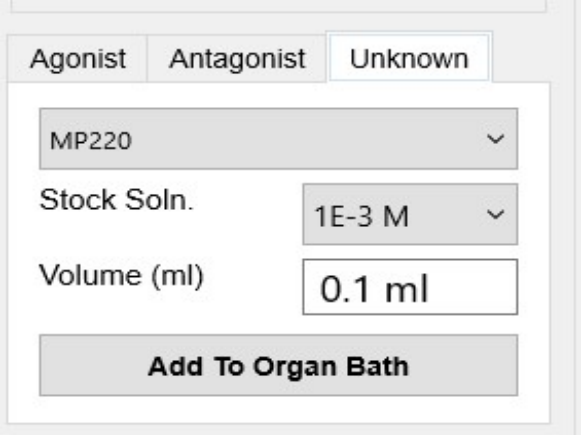
Antagonist	**Receptor**
Mepyramine	H_1 histamine receptors
Atropine	Muscarinic cholinoceptors
Tubocurarine	Nicotinic cholinoceptors
Naloxone	Mu-opioid receptors
Prazosin	$Alpha_1$-adrenoceptors
Yohimbine	$Alpha_2$-adrenoceptors
Hyoscine	Muscarinic cholinoceptors
Heparin	Inositol 1,4,5-trisphosphate (IP3) receptor inhibitor

Propanolol	Beta-adrenoceptors
Nifedipine	Calcium channels blocker
Thapsigargin	sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) inhibitor

Adding an Unknown Drug

To add an unknown drug drug to the organ bath or Krebs' solution reservoir:

a) Select the **Unknowns** page.



b) Select the drug to be applied from the list of available unknown drugs.

c) Select the concentration of the drug solution to be applied from the **Stock Soln.** list.

d) Enter the volume (between 0 and 1 ml) of the stock solution to be applied into the **Volume** box.

e) Select **Organ Bath** from the **Add to l**ist if the antagonist is to be applied directly to the organ bath. Select **Reservoir** if the drug is to be added to the solution reservoir.

f) Click the **Add to** button to inject the volume of the selected stock solution agonist into the organ bath or reservoir.

Calculating the Final Bath Concentration (organ bath)

When adding drugs to the organ bath, the final bath concentration (FBC), in M (Moles/litre)., is related to the stock solution concentration [stock] (in M) the volume of stock added to the bath (in ml) and the bath volume (ml) by the formula,

$$[FBC] = \frac{[stock] \times \text{Volume added}}{\text{Bath volume}}$$

To determine the volume to add to achieve a specified FBC:

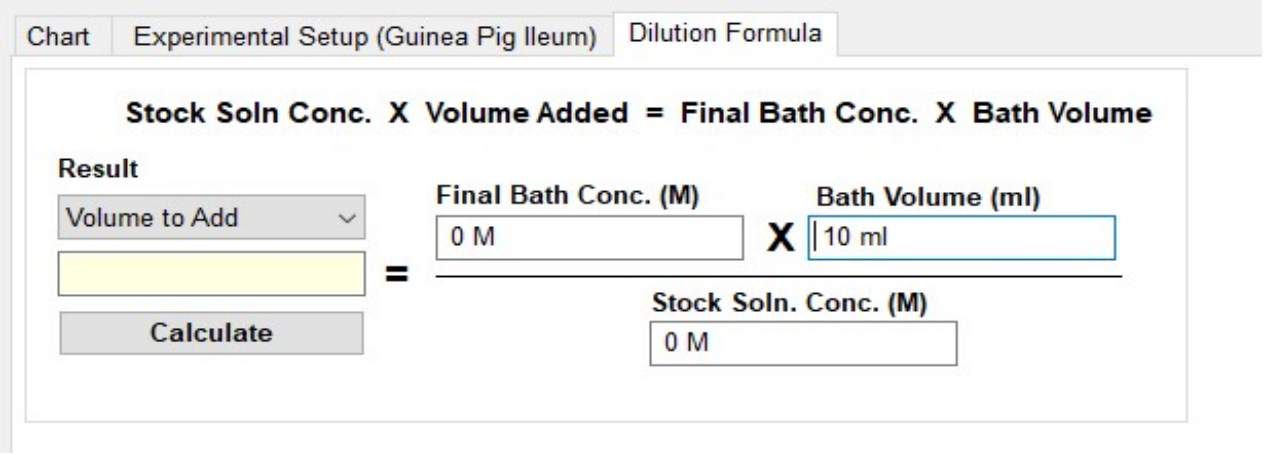
a) Select the **Dilution Formula** page and **Volume to Add** as the Result.

b) Enter the bath volume (**10 ml)** into the **Bath Volume** box,

c) Enter the required FBC in the **Final Bath Conc** box.

d) Enter the selected stock solution concentration in the **Stock Soln. Conc**. box.

e) Click the **Calculate** button.



Calculating the Final Bath Concentration (reservoir)

When adding drugs to the reservoir, the final bath concentration (FBC), in M (Moles/litre)., is related to the stock solution concentration [stock] (in M) the volume of stock added to the reservoir (in ml) and the reservoir volume (ml) by the formula,

$$[FBC] = \frac{[stock] \times \text{Volume added}}{\text{Reservoir volume}}$$

To determine the volume to add to achieve a specified FBC:

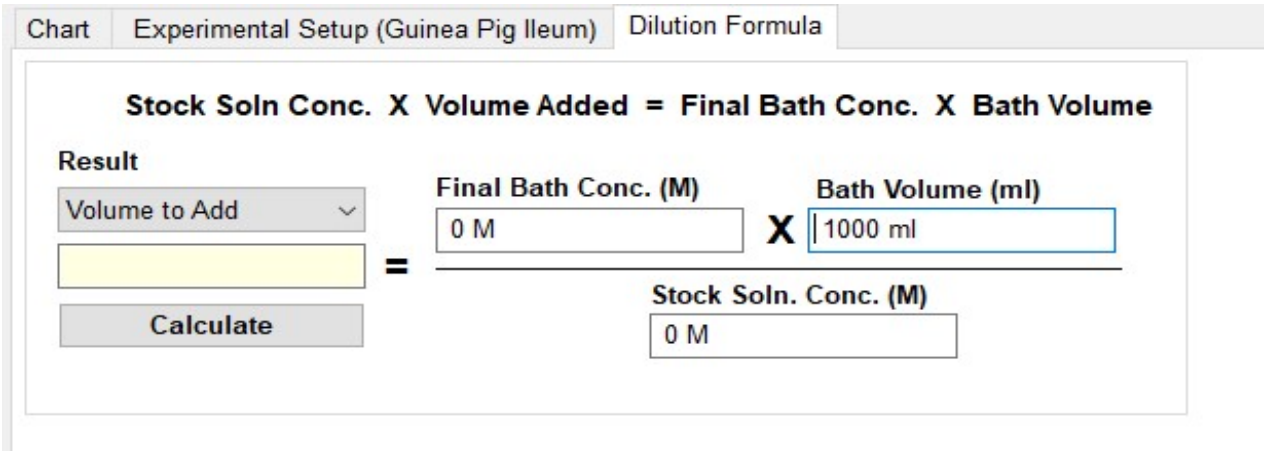
a) Select the **Dilution Formula** page and **Volume to Add** as the Result.

b) Enter the reservoir volume (**1000 ml)** into the **Bath Volume** box,

c) Enter the required FBC in the **Final Bath Conc** box.

d) Enter the selected stock solution concentration in the **Stock Soln. Conc**. box.

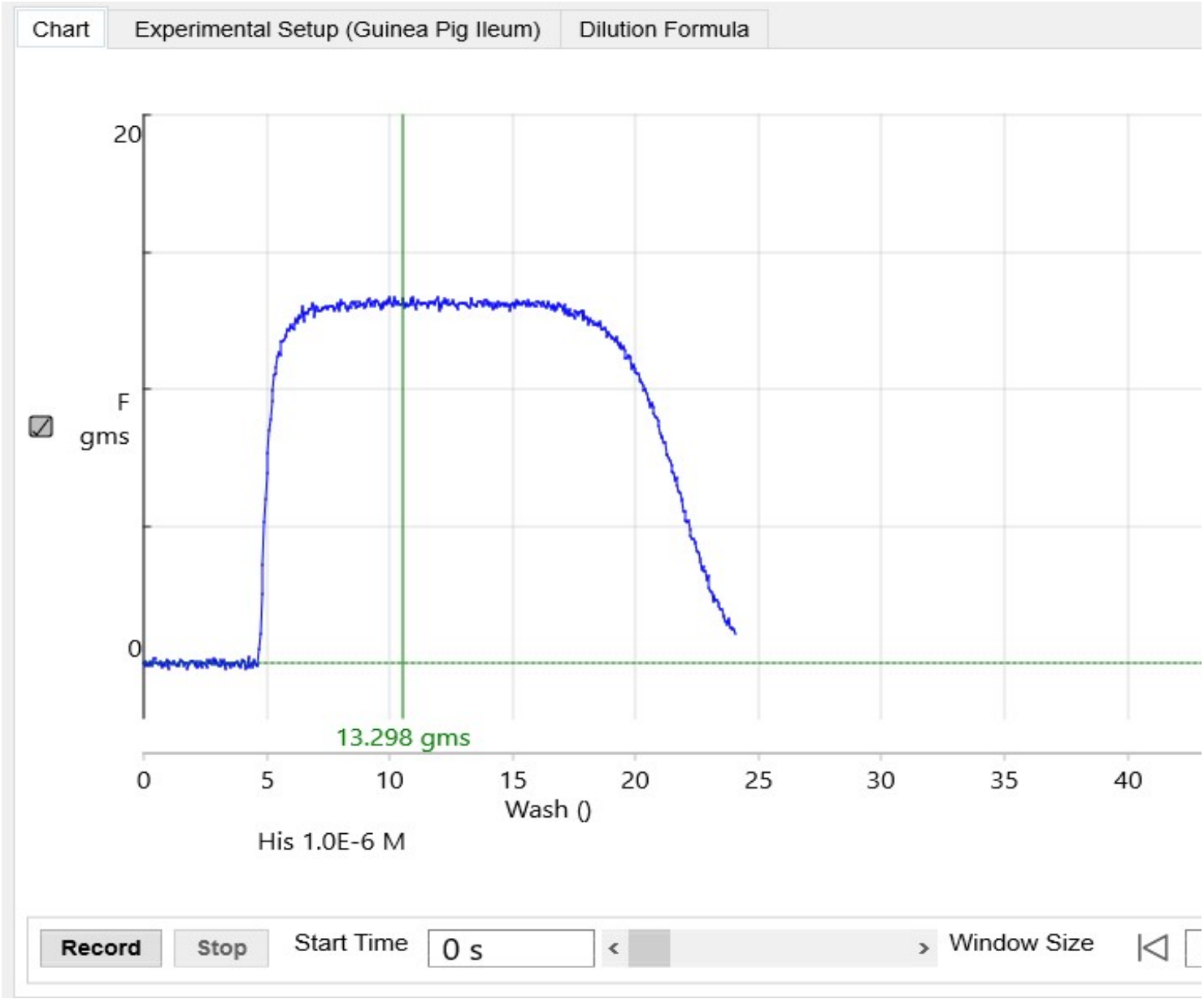
e) Click the **Calculate** button.



Measuring Tissue Responses

To measure the peak amplitude of tissue contractions:

a) Click the **Stop** button to stop recording.



b) Using the scroll bar at the bottom of the chart display, select a section of the recording containing the tissue contraction to be measured. You can also adjust the size of the display window by entering a new duration into the **Window Size** box or expand or contract the window using the arrow buttons.

c) Drag the measurement cursor on the chart display to the point on the recording trace to be measured (the peak of nerve stimulated responses, or the plateau of agonist responses). The contractile force at the cursor point (in units of gms.) is displayed below the cursor.

Menu Options

Printing a chart recording

To print a copy of the displayed chart recording on a printer, select **Print** from the **Fil**e menu.

Copying the chart recording to other programs

To copy the data points of the displayed chart recording to the Windows clipboard for pasting into a spreadsheet or graph plotting program, select **Copy Data** from the **Edit** menu.

To copy the a picture of the displayed chart recording to the Windows clipboard for pasting into a Word document or a PowerPoint presentation, select **Copy Image** from the **Edit** menu.

S**aving a chart recording to file**

To save the current chart recording for the current experiment to a data file, select **Save Experiment** from the **File** menu and enter the name of a new data file.

Loading an existing chart recording from file

To load a previously saved chart recording from a data file, select **Load Experiment** from the **File** menu and enter the name of the data file.

References

The OBSIM simulations are based upon the experiment results reported in the following studies, some of which are by the originators of the methods.

Guinea pig ileum

Paton W.D. (1957) The action of morphine and related substances on contraction and on the acetylcholine output of coaxially stimulated guinea-pig ileum. Brit. J. Pharmacol. 11, 119.

Barker L.A (1985) Regional variation in the sensitivity of longitudinal smooth muscle to histamine at H1-receptors in guinea pig ileum and colon. Br. J. Pharmacol. 85, 377-381

Chick biventer cervicis

Ginsborg B.L. & Warriner J. (1960) The isolated chick biventer cervicis nerve-muscle preparation. Brit. J. Pharmacol. 15, 410.

Harvey A.L. & Marshall I.G. (1977) The actions of three diaminopyridines on the chick biventer cervicis muscle. European Journal of Pharmacology, 44 303-309.

Marshall I.G. (1971) Actions of acetylcholine and carbachol on the chick biventer cervicis muscle. Br. J. Pharmacol. (1971), 42, 462472.

Rat phrenic nerve diaphragm

Bulbring, E. (1946) Observations on the isolated phrenic nerve diaphragm preparation of the rat. Brit. J. Pharmacol. (1946), 1, 38-61.

Meyler W.J., Wesseling H. and Agoston S. (1976) The effects of dantrolene sodium on cardiac and skeletal muscle in rats, European J. Pharmacol. 39 127--131.

Gibb A.J. & Marshall I.G. (1986) Nicotinic antagonists produce differing amounts of tetanic fade in the isolated diaphragm of the rat. Br. J. Pharmacol. 89, 619-624

C.B. Ferry C.B. (1988) The origin of the anticholinesterase-induced repetitive activity of the phrenic nerve-diaphragm preparation of the rat in vitro. Br. J. Pharmacol. 94, 169-179

Nguyen-Huu T., Molgo J, Servent D. & Duvaldestin P. (2009) Resistance to D-Tubocurarine of the Rat Diaphragm as Compared to a Limb Muscle Influence of Quantal Transmitter Release and Nicotinic Acetylcholine Receptors. Anesthesiology 110:1011–5

Jenkinson D.H. (1957) The nature of the antagonism between calcium and magnesium ions at the neuromuscular junction. J. Physiol. I38, 434-444

Rabbit jejunum

Bowman W.C. & Hall M.T. (1970) Inhibition of rabbit intestine mediated by alpha- and beta-adrenoceptors. Br. J. Pharmacol. 38, 399-415.

Finkleman B. (1930) On the nature of inhibition in the intestine. J. Physiol. 70,2, 145-157.

Walker R.L. & Scott C.C (1989) Use of the Rabbit Intestine in Smooth Muscle Pharmacology Experiments: A New Approach in TESTED STUDIES FOR LABORATORY TEACHING: Proceedings of the Ninth Workshop/Conference of the Association for Biology Laboratory Education (ABLE).

Rabbit aortic rings

Kalsner S. (1971) Mechanism of potentiation of contractor responses to catecholamines by methylxanthines in aortic strips. Br. J. Pharmac. 43, 379-388.

Bernard J. Norman B.J. & Leathard H.L. (1990) Evidence that an atypical beta-adrenoceptor mediates the inhibition of spontaneous rhythmical contractions of rabbit isolated jejunum induced by ritodrine and salbutamol. Br. J. Pharmacol. 101, 27-30

Acknowledgements

OBSIM was developed in the Dept. of Physiology and Pharmacology (now part of the Strathclyde Institute for Pharmacy & Biomedical Sciences) at the University of Strathclyde, starting in 1996. Thanks are particularly due to Drs. Christopher Prior, Edward Rowan, Charles Kennedy & Robert Drummond, who conceived the need for the simulations as a part of their teaching programs, specified aims and objectives, selected the drugs and receptor systems to be covered, and evaluated the accuracy of the simulations.

It is worth also remembering the fundamental contributions of the original developers and promoters of these experimental models and methods who contributed so much to the creation of the science of pharmacology, notably William Paton (guinea pig ileum), Edith Bulbring (rat diaphraghm), Bernard Ginsborg (chick biventer cervicis) & B. Finkleman (rabbit jejunum).