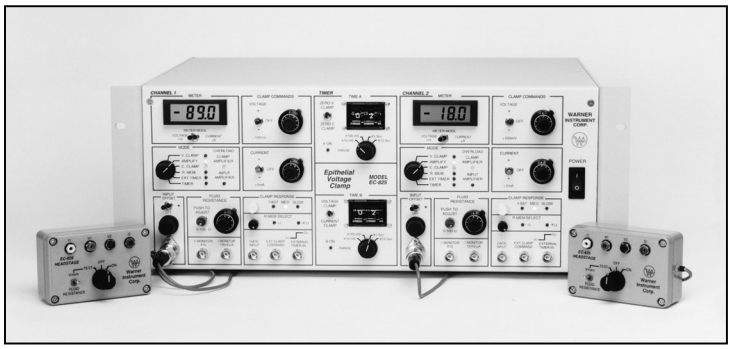
Warner Instruments, Inc.
Epithelial Voltage Clamp
Models EC-825 & EC-825LV



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The **EC-825** and **EC-825LV** Epithelial Voltage Clamps from Warner Instruments provide accurate measurements of transepithelial voltage, short circuit current and membrane resistance. Important features include fluid resistance compensation, membrane resistance readout, high voltage compliance and small water tight headstages. Operating modes include voltage clamp, current clamp, voltmeter and resistance.

Unique Features Include

- ✓ **Membrane Resistance Measurement:** Accurate resistance measurements are made with the membrane mounted in the chamber. The measurement is made using a low frequency bipolar signal to avoid polarization of the membrane [ideal for monolayers]. Resistances up to 200 kΩ are displayed on the meter with push button convenience.
- ✓ **Clamp Speed Selection up to 10 µs:** Three clamp speeds provide optimum recording conditions for a variety of applications. In Fast mode, preparations with low access resistance (small tissues or monolayers) can be clamped with speeds up to 10 µs. Typical Ussing chambers with larger tissues will use Medium or Slow modes for stable, oscillation free clamping.
- ✓ **Independent Voltage and Current Commands:** Internal command controls are provided for both voltage clamp and current clamp modes.
- ✓ **Watertight Headstage with Model Membrane:** The small, compact headstage can be located close to the measurement site to keep input leads short for reduced noise pick-up. The model membrane circuit simulates a preparation to provide convenient operational checks of the clamp. Internal circuits are protected against the invasion of corrosive saline solutions by a watertight seal.
- ✓ **Choice of Compliance Voltage:** The high voltage compliance of the **EC-825** (±120 V) is important for studies of low resistance [leaky] epithelial cells and in applications in which long agar leads in the current passing circuit produce large voltage drops which must be compensated. Additionally, high compliance aids in charging large membrane capacitances typical of epithelial tissues, resulting in faster settling times and improved overall clamp performance. Studies with small tissue samples or monolayers in set-ups with low access resistance may not require high compliance. For these applications, the **EC-825LV** (±15 V) offer both a lower cost and a safer environment for the membrane.

Additional Features Include

- ✓ **High CMR:** Differential voltage recordings are made with very high common mode rejection providing accurate measurements free from the effects of common mode potential changes of a noisy environment.
- ✓ **Onboard Timer Controller:** The **EC-825** include two event timers to provide cycle times up to 2000 seconds. Times are set using 2 digit thumbwheel switches and 4 position range switches. Once set, the timer will free run, eliminating the need for a computer or other external device to control the experiment.
- ✓ **External Instrument Control:** The clamp can be operated by an external programmer, lab timer or computer. Logic control of clamp mode and clamp command levels is possible as well as simultaneous mixing of external linear commands.

NOMENCLATURE

Text conventions

This manual refers to amplifier controls at four functional levels; operational sections, control blocks, specific controls within a block, and settings of specific controls. To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instruments product numbers are presented using **bold type**.
- References to instrument panel operational sections are specified using *ITALICIZED UNDERLINED CAPS*. (e.g., *CHANNEL 1*, *TIMER A*)
- References to instrument panel control blocks (within operational sections) are specified using UNDERLINED SMALL CAPS. (e.g., METER, CLAMP COMMANDS)
- References to specific controls within a block are specified using NON-UNDERLINED SMALL CAPS. (e.g., MODE SWITCH, TIMER RANGE)
- Finally, references to individual control settings are specified in *italic type*. (e.g., *Amplify*, *100 mV*)
- Input connections to the headstage are printed in a **bold type**. (e.g., **I1 output**, **V2 input**)
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS

CONTROL DESCRIPTION

The following is a description of the operating controls, inputs, and outputs located on the front and rear panels of the instrument.

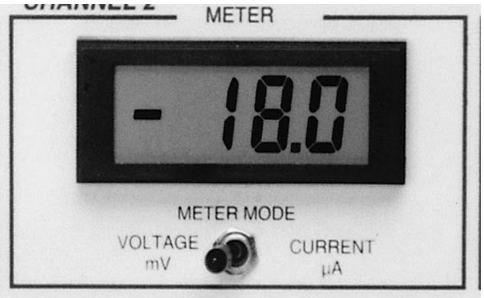
The front panel is comprised of two channel sections separated by a timer section. Each channel section (*CHANNEL 1*, *CHANNEL 2*) is further subdivided into control blocks termed METER, MODE, CLAMP COMMANDS, INPUT OFFSET, FLUID RESISTANCE, and CLAMP RESPONSE.

The *TIMER* section is divided into TIME A and TIME B subsections.

Channels 1 and 2 - Control blocks

Meter

The METER section is comprised of a 3.5 digit LCD and 2 position toggle switch (METER MODE).

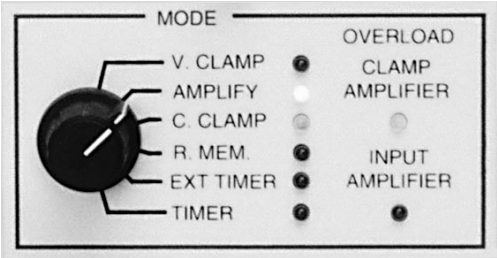


The METER MODE switch selects between *current* and *voltage* readings. Full scale is ±1,900 µA and ±199 mV for current and voltage positions, respectively. Either *current* or *voltage* can be displayed for any selection within the MODE control block, with the exception of R MEM mode.

In R MEM mode, the meter reads the membrane resistance in either Ω or kΩ, as selected by the R MEM SELECT push button in the CLAMP RESPONSE control block.

Mode

This block is comprised of a six position MODE SELECTOR switch and various LED indicator lights. The MODE SELECTOR is used to select among the various functions within its respective *CHANNEL*.



LED's indicate which MODE has been selected. The meaning of available MODE selections are shown on the next page.

Moreover, two LED's are provided to indicate overload conditions in the clamp amplifier or input amplifier circuitry.

Available MODE selections are:

V Clamp	Selects voltage clamp mode.
Amplify	With the exception of *fluid resistance* measurements, the clamp amplifier is disconnected from the headstage resulting in no signal at the HEADSTAGE **I1 output**. This is a true amplify operation.
C Clamp	Selects current clamp mode.
R MEM	Selects membrane resistance measurement mode. A constant current, 2 Hz square wave is injected and the resultant voltage is measured across the membrane. The calculated resistance is displayed on the meter.
Ext Timer	External timer mode. A TTL signal applied to the EXTERNAL TIMER IN BNC will switch the amplifier between current clamp (TTL=*high*) and voltage clamp (TTL=*low*) modes.
Timer	Internal timer mode. The programmable internal timer switches between current clamp and voltage clamp modes.

Input Offset

The toggle switch selects input polarity (+ or -) or *off* (no offset). The ten turn control provides input offset adjustment from *0-120 mV*. The HEADSTAGE (for this *CHANNEL)* connects to the **EC-825** in this control block.

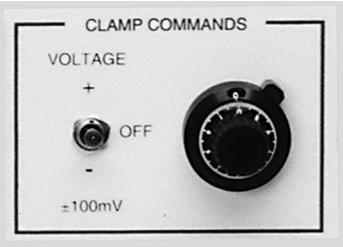
Fluid Resistance

This control is used to compensate for any voltage drops due to the intrinsic resistance of the bathing solutions. A 25 µA current (this value can be adjusted internally) is injected at the HEADSTAGE **I1 output**. The measurement/adjustment is made by depressing the PUSH TO ADJUST button and turning the ten turn dial until *zero* is read on the digital meter. The fluid resistance is read from the dial (*0-100 Ω*). NOTE: This range can be extended to 1 kΩ, *see* the Appendix for details.

MONITORS

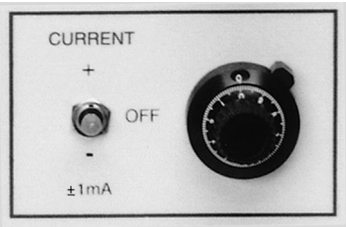
V Monitor x10	Output is the differential voltage (V1-V2) multiplied by 10.
I Monitor 10mV/µA	Output voltage is proportional to the current flowing through the preparation.

Clamp Commands



Voltage

This block controls the holding potential effective in voltage clamp mode except when the voltage clamp is activated by TIMER A. The operational range is adjustable from *zero* to *±100 mV* with the polarity selected by toggle switch.

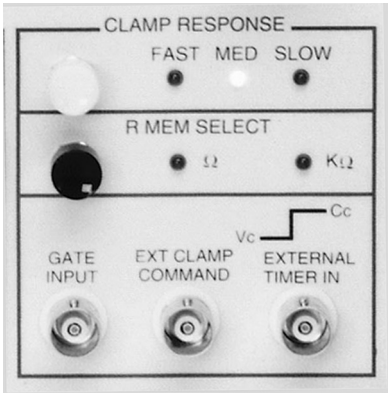


Current

This block controls the holding current effective in current clamp mode except when the current clamp is activated by TIMER A. The operational range is adjustable from *zero* to *±1 mA* with the polarity selected by toggle switch.

NOTE: A TTL=*high* logic level at the GATE INPUT BNC (*see* below) will disable the CLAMP COMMANDS control block.

Clamp Response



CLAMP RESPONSE: Selects *fast*, *med* or *slow* clamp speeds. This control is active in both current and voltage clamp modes.

R MEM SELECT: Selects the membrane resistance measurement range (Ω or kΩ). A constant current bipolar square wave is passed through the membrane (10 µA in the 0-2 kΩ range and 1 µA in the 0-100 kΩ range) and the membrane resistance read on the panel meter which scales automatically with the range selected.

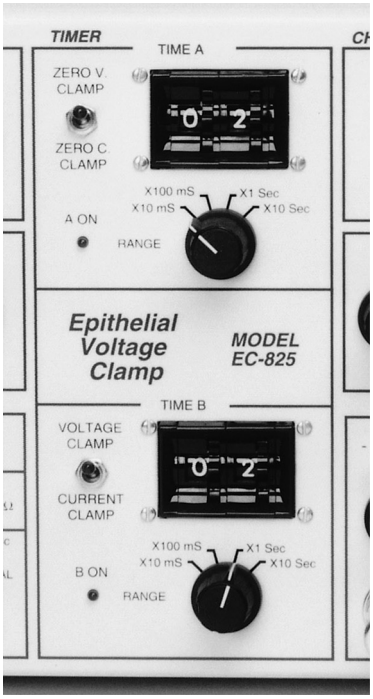
EXTERNAL INPUT BNC's :

GATE INPUT: All clamp commands (current, voltage and external) are deactivated with a TTL=*high* signal applied. Used to select *zero current mode*.

EXT CLAMP COMMAND: An external voltage or current clamp command input. Applied current or voltage commands are summed with their respective counterparts in the CLAMP COMMANDS control block. In voltage clamp mode, the ratio of EXT CLAMP COMMAND voltage to applied clamp voltage is 0.01 V/mV. In current clamp mode the ratio of EXT CLAMP COMMAND voltage to applied clamp current is 10 mV/µA. This input is inactive when TIMER A is *on* or when GATE INPUT is *high*.

EXTERNAL TIMER IN: Activated by selecting *Ext Timer* in the MODE control block. A TTL signal at this BNC switches the amplifier between voltage clamp (TTL=*low*) and current clamp (TTL=*high*) modes.

Timer - Control blocks



The internal timer of the **EC-825** is used to program one or both channels by selecting *timer* in the MODE control block. Times are set using two thumb wheel digits and a multiplier switch. Times can be set from *10 ms* to *990 seconds* in 4 ranges (*x10 ms, x100 ms, x1 sec,* and *x10 sec*).

TIME A sets the 'off' time or *zero clamp* time. The associated toggle switch provides for selection of *zero v clamp* or *zero c clamp* modes. TIME A on condition is indicated by a lit *a on* led.

TIME B is used to set the 'on' time for either *voltage clamp* or *current clamp* modes as selected with the associated toggle switch. TIME B on condition is indicated by a lit *b on* led.

Rear panel description

The instrument rear panel provides several connections for control input and data outputs.

Line power connector and fuse

The power cord connects to the power connector. Check the serial number tag on the rear panel to be sure that the instrument is wired for the operating voltage used. The fuse (3AG type, regular blow) used in the **EC-825** will depend on the line voltage; 1/2 amp for 100 or 130 VAC, and 1/4 amp for 220 or 240 VAC. Replacement should only be made with the proper ampere rating.

I/O port

This 25 pin connector allows the **EC-825** to be connected for computer operation. Pin designations are:

1	V MONITOR x 10, Ch. 1	10	TIMER B
2	I MONITOR, Ch. 1	11	SYNC OUTPUT
3	EXT CLAMP COMMAND, Ch. 1	14	V MONITOR, Ch. 2
4	GATE INPUT, Ch. 1	15	I MONITOR, Ch. 2
5	EXTERNAL TIMER IN, Ch. 1	16	EXT CLAMP COMMAND, Ch. 2
6,7 & 8	circuit ground	17	GATE INPUT, Ch. 2
9	TIMER A	18	EXTERNAL TIMER IN, Ch. 2

TA and TB BNC's

These are BNC outputs from the *TIMER* section. TA refers to the TIMER A control block and TB refers to the TIMER B control block. A *+15 V* signal indicates *on* status and a *0 V* level indicates *off* status for the respective *TIMER* block.

Sync output

Useful for synchronizing an oscilloscope with the **EC-825** while monitoring output signals. Connects to oscilloscope trigger input.

Grounds

Both circuit (black) and chassis (green) grounds are provided at rear binding posts. The instrument is shipped with the two grounds connected *via* a shorting bar, If needed, this shorting bar can be disconnected allowing separation of the grounds. Separating these grounds can, in some cases, result in a lowering of the 60 Hz noise level, however, experimentation will determine which is best for each set up.

Additional components and comments

Connecting to line power

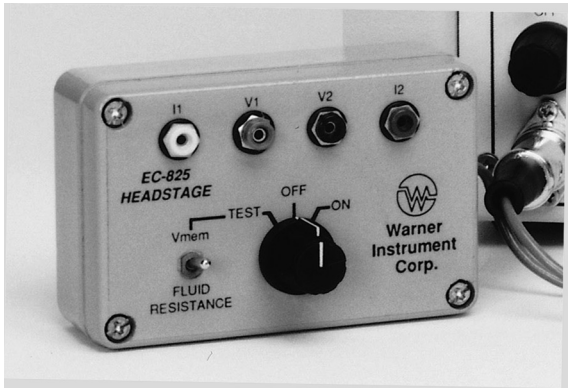
The model **EC-825** is supplied with a 3-conductor line cord. One conductor provides a connection between the instrument housing and the power system earth ground. Safe operation of this instrument will be assured provided that the power outlet is wired correctly and is connected to earth. If the ground pin of the line cord is removed for any reason the instrument chassis must be connected to earth ground using a separate heavy gauge (14 or larger) ground wire.

High voltage outputs

CAUTION: The clamp is capable of high power output (±120 V @ 10 mA). When handling a HEADSTAGE **I1 electrode cable**, be sure to set the MODE SELECTOR switch to *amplify* and the HEADSTAGE SELECTOR switch to *off*.

Headstage

Each channel of the **EC-825** is provided with a matched headstage. Each headstage is has inputs for the **I1**, **V1**, **V2**, and **I2 electrode cables** and a three position switch selecting *on*, *off* or *test* modes. Furthermore, *test* mode has a two position toggle switch selecting for *Vmem* or *fluid resistance*.



Moreover, each headstage is equipped with a built-in model membrane allowing for a rapid and convenient instrument test. (*See* page 17 for test procedure).

Connecting the headstages

The headstage connects to the instrument via an 8-pin DIN connector in the INPUT OFFSET control block. To reduce noise the headstage should be placed as close to the test site as possible.

IMPORTANT: Headstages are matched to each channel and are marked L (left, *Channel 1*) and R (right, *Channel 2*) on their serial number label.

SETUP AND INITIAL TEST

The purpose of this section is twofold. First, a description of the equipment needed to measure the basic electrical properties of an epithelium is provided. Second, a series of procedures are provided to test the performance of the instrument.

Equipment

The equipment required for the study of any epithelium (including tissue cultured epithelia) can be divided into three components. The first component is a pair of hemi-chambers (typically termed Ussing chambers) in which the epithelium acts as a partition between the two chambers. Thus the epithelium separates two compartments; the mucosal or luminal compartment and the serosal or blood side compartment. The second component contains the electrodes for sensing the epithelial voltage and for passing current and the third component is comprised of the instrumentation necessary for measuring both voltage and current.

Chambers

Irrespective of the detailed design, useful chambers must have several common features. These include:

i. Temperature control: The chamber must have provisions for controlling the temperature of the mucosal and serosal bathing solutions and for aerating both solutions with the gas mixture of choice.

ii. Damage control: The chamber must have a design which minimizes damage to the cells which contact the inner circumference of the hemi-chamber. If not protected against, such damage (termed edge damage) will result in a measured epithelium resistance which is lower than the native tissue.

iii. Support: The epithelium must be supported on one side by a rigid but permeable structure. This reduces epithelial stretch and the possibility of cell or tight junction damage.

iv. Voltage electrodes: The voltage measuring electrodes must be placed as close as possible to the epithelium. This will reduce the magnitude of the solution series resistance which, if large, can compromise the ability to precisely voltage clamp the epithelium

v. Current electrodes: The current passing electrodes must the placed in the rear of the chambers and as far as possible from the epithelium. This will assure a uniform current density across the epithelium. A non-uniform current density will result in an overestimate of the epithelial resistance.

vi. Solution changes: Bathing solutions must be easily and rapidly changed without interrupting electrical measurements and without altering the electrical properties of the epithelium.

vii. Multi-functionality: Ideally, the chamber should be multi-functional. Thus, in addition to measuring transepithelial electrical properties, it should also be constructed to allow for the use of micro- or ion selective electrodes. The former electrodes are essential for determining the individual membrane resistances, while the latter are required to determine membrane ionic permeabilities.

viii. Additional features might include the ability to measure cell volume and use intracellular fluorescent dyes. Two chamber designs currently in use for the study of epithelial transport are shown below.

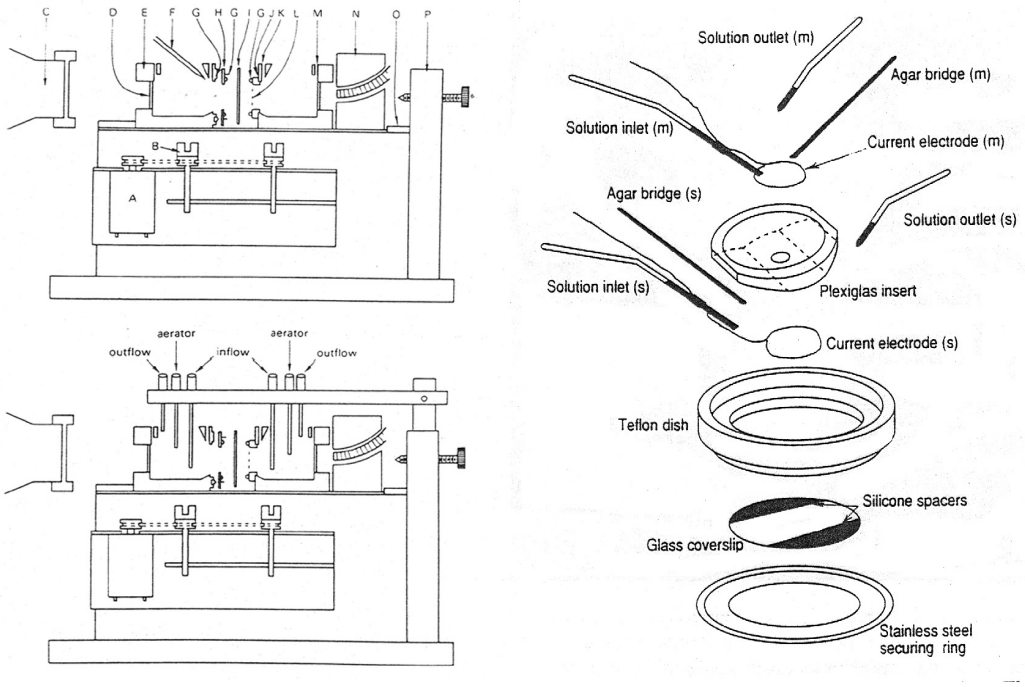


Figure 1. Two basic chamber designs for studying epithelial transport. Left: this is the original design by Ussing. This chamber circulates solution across the surface of the epithelium using a gas lift system which also aerates the ringers and can be temperature controlled. A disadvantage of this system is that it is closed and thus does not meet criteria vi and vii. In addition the original design caused significant edge damage (see ii). Right: this chamber is designed to be used on an inverted microscope and meets all of the listed criteria including the ability to measure transepithelial electrical properties and monitor intracellular ionic activities using fluorescent dyes. A disadvantage of this design is that solutions must be continuously flowed through the chamber and that this solution must be preheated.

Electrodes

Electrodes are an essential component of any electrophysiological set-up since they provide the low resistance interface between the Ringer's solution and the electronic equipment. This section considers the electrodes used to sense the epithelial voltage and to pass a transepithelial current. Although it may seem trivial, careful choice the electrodes used is important. Some guidelines are listed below.

Choices: One has three choices in voltage measuring electrodes. These are silver/silver chloride (Ag/AgCl) electrodes, calomel electrodes or agar bridges. Which electrode set to use? The answer to this question depends upon the epithelium to be studied, as well as the composition of the solutions bathing the epithelium.

i. Silver/silver chloride (Ag/AgCl) electrodes can only be used if the epithelium is not sensitive to trace levels of Ag^+ ions (e.g., toad urinary bladder epithelium is very sensitive to trace levels of Ag^+) and if the Cl^- concentration (activity) in the solutions bathing both sides of the epithelium are identical. Unequal Cl^- concentrations will result in an asymmetry voltage difference between the voltage measuring electrodes. This asymmetry voltage will be summed into the transepithelial voltage yielding a voltage artifact (i.e., the measured voltage will be high or low depending upon the ratio of mucosal to serosal Cl^- concentrations).

ii. If the epithelium is sensitive to Ag^+ or is bathed by solutions containing different Cl^- concentrations, then agar bridges connected to either Ag/AgCl electrodes or calomel electrodes must be used. The advantage of the agar bridge-Ag/AgCl electrode combination compared to calomel electrodes is that they are small and easy to locate close to the epithelial surface. Moreover, they are inexpensive. Agar bridge electrodes must be connected *via* a salt solution to either the Ag/AgCl or calomel electrodes which are connected in turn to the **EC-825**. The same electrode configurations can be used for the current passing limb of the electronics.

Fabrication: Sintered Ag/AgCl pellet electrodes are commercially available from Warner Instruments. Call our offices or see our catalog for our extensive selection. An alternate choice is to use silver wire which has been chlorided by the user. (*See* Appendix for techniques on chloriding Ag wire).

Agar bridges are easily constructed by heating a mixture of 5% agar with 1M KCl (w/v). While still hot, the agar can be drawn into polyethylene tubing using a syringe or vacuum line. Since the polyethylene tubing is opaque, it is convenient to add a dye (e.g., methylene blue) to the agar/KCl solution. This allows the visualization of any discontinuity in the agar bridge which can cause an overload (lights and audio signal) from the input and/or clamp amplifier.

CAUTION: Current passing electrodes must have a low interface resistance with the solution to work properly.

If the interface resistance is too large, it will limit the current passing capacity of the electronic equipment. Ideally, use of a Ag/AgCl sheet or wire coil in the rear of each hemi-chamber is sufficient to assure a uniform current density across the epithelium. However, if the tissue is sensitive to trace levels of Ag^+, a wide bore agar bridge placed at the rear of each hemi-chamber will be required to make a connection between the electrodes and solution. If this technique is necessary, then every effort should be made to keep the length of the bridge short and to fill the bridge with a low resistance solution to maximize the current passing capability of the electronic equipment. A disposable pipette tip

partially filled with agar, back filled with a low resistance solution (1M KCl) and coupled to the electronic equipment with a Ag/AgCl wire or sintered pellet works very well.

Electronics

Four important features of an epithelial clamp are described in this section. They are compliance voltage of the current passing amplifier, speed of the current and voltage circuits, series resistance compensation and internal pulse generation.

Compliance voltage of the current passing amplifier (defined as the maximum voltage output of the amplifier) is important if agar leads are used as part of the current passing circuit. Some clamps use amplifiers which have a compliance voltage of 10 volts. If the total resistance of the current passing circuit is 10 kΩ, this means that the maximum current the clamp can pass is +1.0 mA. Although this current level is sufficient for high resistance (tight) epithelia, it most likely will be marginal for low resistance (leaky) epithelia or for epithelia whose resistance has been decreased due to an experimental maneuver. The **EC-825** uses a 110 V current passing amplifier making this an instrument of choice for leaky epithelia. The **EC-825** has outputs which can be easily connected to chart recorders, oscilloscopes or computers.

Minimum equipment requirements are:

i. A current/voltage clamp amplifier: This is essential to record the transepithelial voltage, the transepithelial resistance and the short circuit current (I_{sc}).

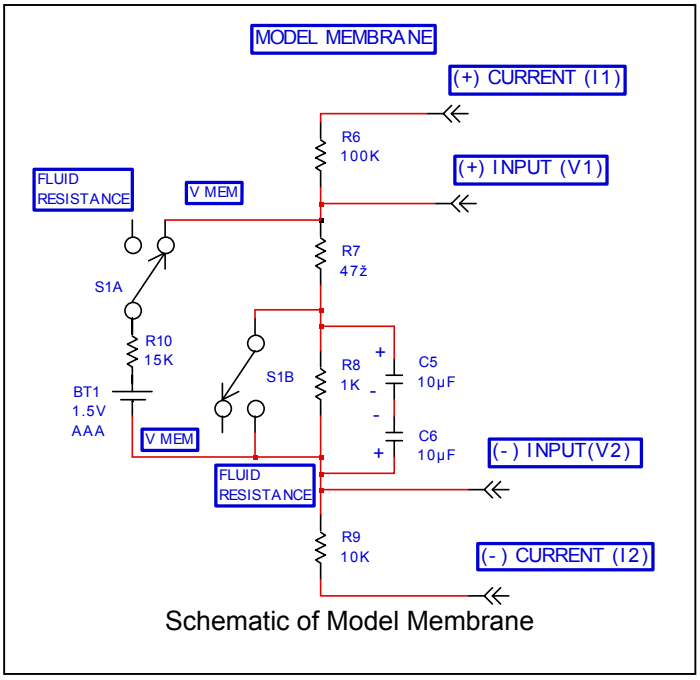
ii. A pulse generator: This device is necessary to either pass a current across the epithelium and record the change in the transepithelial voltage, or to voltage clamp the epithelium and record the current passed to maintain that voltage. Either of these two measuring schemes allows the calculation of the transepithelial resistance.

iii. A computer with acquisition hardware and software: A well chosen acquisition system can simultaneously display several data channels while digitizing and storing the data in memory for later viewing and analysis. An advantage of using a computer interfaced with the current/voltage clamp is that the data can be immediately analyzed providing feedback on the viability of the epithelium.

iv. If a computer is unavailable, then a 2-4 channel paper chart recorder and oscilloscope is necessary. The chart recorder will provide a permanent record of the experiment and should have a pen speed fast enough to record changes in transepithelial voltage or current produced by the pulse generator. An oscilloscope is required to display the time dependent changes in transepithelial voltage during a current pulse. In addition, the oscilloscope can be used to determine whether the current/voltage clamp is stable (i.e. it is not oscillating) as well as the response time of the clamp (e.g., is the current or voltage pulse that is being passed square or does it have a finite rise time?). In the **EC-825,** the speed of the clamp (i.e. rate of rise of the current pulse) can be selected on the front panel. A square current pulse is important when estimating epithelial surface area using capacitance measurements (*see* page 26).

Quick test

Each **EC-825** headstage contains a model membrane which can be made functional by selecting the *test* position on the HEADSTAGE SELECTOR switch.



Schematic of Model Membrane

Initial control settings

- Set the HEADSTAGE SELECTOR switch to *off*.
- Set the METER MODE switch to *voltage (mV)*.
- Set the MODE SELECTOR switch to *amplify*.
- Set polarity switches for VOLTAGE COMMAND, CURRENT COMMAND and INPUT OFFSET to their respective *off* positions.

After the instrument has warmed up, the digital voltmeters should show a potential close to
0 mV. Completion of the following procedures verifies the proper operation of the voltage clamp.

Monitoring model membrane voltage

- Set the HEADSTAGE SELECTOR switch to *test* and the TOGGLE switch to *Vmem*.

The digital panel meter should show a potential in the neighborhood of *100±10 mV,* depending on the condition of the battery inside the headstage.

Checking zero voltage clamp

- Change the MODE SELECTOR switch to *v clamp*.

The panel meter should read *0 V*. The membrane is now clamped to zero volts.

Check voltage clamp command

- If the VOLTAGE COMMAND TOGGLE switch is moved up or down (+ or - selected), the 10-turn dial will now voltage clamp the membrane at voltages other than zero volts. These new clamping voltages should be displayed on the panel METER.
- Return the VOLTAGE COMMAND TOGGLE switch to *off*.

Checking clamp current on panel meter

- Set the METER MODE switch to *current µA*.

The meter now displays the clamp current necessary to clamp the membrane to zero volts.

Fluid Resistance compensation

- Set the MODE SELECTOR switch to *amplify*.
- Set the METER MODE to *voltage (mV)*.
- Set the HEADSTAGE SELECTOR switch to *test* and the TOGGLE switch to *fluid resistance*.
- Press the PUSH TO ADJUST button in the FLUID RESISTANCE control block.

The LCD meter should display a *non-zero* value.

- While depressing the PUSH TO ADJUST button, advance the FLUID RESISTANCE POTENTIOMETER until the meter again reads *zero*.

The 10-turn dial on the potentiometer should read approximately *47 Ω* (the value of the dummy fluid resistor).

- Switch the METER MODE switch to *current (µA)*.
- Press the PUSH TO ADJUST button in the FLUID RESISTANCE control block to check the amplitude of the fluid resistance test current, it should be set for *25 µA*.

OPERATION

Procedures are presented for using this experimental set-up to measure the basic electrical properties of epithelia including the transepithelial potential difference, the transepithelial resistance, short circuit current and capacitance.

Connecting the EC-825

This section describes the steps involved in connecting the **EC-825** to an epithelium.

From epithelium to headstage

- Start with the instrument power *off*.
- Set the MODE SELECTOR switch to *amplify*.
- Set the HEADSTAGE SELECTOR switch *off*.
- Place the voltage measuring and current passing electrodes into the chamber.
- Connect the electrodes to the HEADSTAGE **inputs** using the provided color coded leads. WARNING: Electrodes connected to **V1** (voltage electrode of side 1) and **I1** (current electrode of side 1) must be placed in the same bathing solution. Similarly, **V2** and **I2** must be placed in the same opposing bathing solution.

From the current/voltage clamp to external monitors

The **EC-825** is a completely self contained unit. However, a permanent record of your data can be exported to a paper chart recorder, oscilloscope or computer via the clamp BNC outputs (V MONITOR and I MONITOR in the FLUID RESISTANCE control block).

Checking for asymmetries in the voltage electrodes

Prior to beginning an experiment asymmetries in the voltage measuring electrodes must be compensated.

- Set the HEADSTAGE SELECTOR switch to *off*.
- Select *amplify* on the MODE SELECTOR switch.
- Set the METER MODE toggle to *voltage*.
- Place both voltage measuring electrodes (HEADSTAGE **V1** and **V2 inputs**) into the same bath.
- Turn the HEADSTAGE seLector switch to *on*.
- If the voltage reading on the meter is non-zero then use the INPUT OFFSET POTENTIOMETER to adjust the voltage reading to *zero*.

As a rule of thumb, if the offset voltage is greater than 10 mV, then the electrodes must be replaced and the offset readjusted.

- Set the HEADSTAGE SELECTOR switch to *off*.
- Place the HEADSTAGE **voltage measuring electrodes** into their respective chambers.
- Turn the HEADSTAGE SELECTOR switch to *on* and select the desired measuring mode (see below).

Recording modes

The recording mode to be used (voltage clamp or current clamp) depends upon the experimental design. Advantages and disadvantages of different recording modes are listed below.

Amplify: In this mode the current passing amplifier is disconnected from the current passing electrodes (HEADSTAGE **I1** and **I2 inputs**). This mode is used to measure any asymmetry in the voltage sensing electrodes. Since the current passing amplifier is disconnected, an external voltage or current applied to the EXT CLAMP COMMAND input BNC will not be passed across the epithelium.

Voltage clamp: In this mode the transepithelial voltage is set to a specified value and the amplifier passes a transepithelial current to maintain that voltage. The magnitude and polarity of the clamp voltage is set within the VOLTAGE COMMAND control block. Voltage clamp mode can be used, for example, to determine which ions are actively transported by the epithelium (using radio isotopic flux measurements or pharmacological ion transport blockers).

Current clamp: In this mode the transepithelial current is typically clamped to *zero* (i.e. there is no net transepithelial current flow), the condition the tissue is exposed to *in vivo*. The steady state current can be varied in magnitude and polarity within the CURRENT COMMAND control block. The voltage measured in this mode is produced by the active transport of ions by the epithelium plus the current applied across the epithelium by the current passing amplifier. An advantage of the *zero current* clamp mode that, in the presence of Ag/AgCl wires, the silver contamination of the bathing solutions is minimized.

Resistance of the membrane: In this mode, the **EC-825** automatically determines the membrane resistance by passing a bipolar current pulse of 10 μA (0-2 kΩ range) or 1 μA (0-200 kΩ range). The resistance is read directly from the panel meter in units of Ω or kΩ (e.g., 103 Ω). If the meter displays all zeros, then select the *Ω* setting (by pushing the R MEM SELECT button in the CLAMP RESPONSE control block). If the meter is blank except for a 1 on the far left, then select the *kΩ* setting. If the meter's reading does not change, then either the resistance of the tissue is larger than 50 kΩ or there is a high resistance connection between the headstage and the current or voltage electrodes in the chambers.

External timer: This allows the operator to remotely select between current clamp (TTL=*high*) or voltage clamp (TTL=*low*) modes using either a switching box (not provided) or a computer input connected to the EXTERNAL TIMER IN BNC. In this mode a TTL=*low* signal at the GATE INPUT BNC instructs the EXTERNAL TIMER IN BNC to select between the values set for voltage or current clamp modes. A TTL=*high* signal into the GATE INPUT BNC selects instructs the EXTERNAL TIMER IN BNC to select for *zero current clamp* for current clamp mode or *zero voltage clamp* for voltage clamp mode.

Timer: This setting activates the timer function and allows the operator to either voltage or current clamp the epithelium at zero for a programmed time (set by the THUMBWHEEL switch and RANGE selector of TIME A) and then either current or voltage clamp the epithelium at a preset current or voltage using the clamp command settings for a programmed time interval (set by THUMBWHEEL switch and RANGE selector on TIME B). Four different settings are available in TIMER mode: zero voltage clamp switched to a selected voltage clamp value; zero voltage clamp switched to a selected current clamp value; zero current clamp switched to a selected current clamp value; zero current clamp switched to a selected voltage clamp value. The magnitude and polarity of the selected voltage or current clamp value is set by the calibrated dial and polarity switch of the associated CLAMP COMMANDS.

Warning lights

If the voltage between the HEADSTAGE **V1** and **V2 inputs** exceeds *1.2 V* there will be an audio warning and visual warning that the input amplifier is saturating. Similarly, a saturation of the clamp amplifier has both audio and visual warnings.

Fluid resistance

The resistance between the voltage measuring electrodes is composed of a series combination of the resistance of the epithelium and the resistance of the fluid bathing the tissue (this latter resistance is sometimes called the series resistance). To determine the epithelial resistance the fluid resistance must be measured and subtracted from the total resistance. Moreover, to voltage clamp a tissue at a specified value, then the fluid resistance must be compensated for.

The fluid resistance can be measured and set using one of the following protocols:

Method A

- Connect all electrodes to the chamber and fill with the appropriate Ringers solution in the absence of an epithelium
- Switch the HEADSTAGE SELECTOR switch to *on*.
- Set the METER SELECTOR switch to *voltage*.
- While depressing the PUSH TO ADJUST button in the FLUID RESISTANCE control block, adjust the POTENTIOMETER until the voltage reading on the meter reaches *0 mV*.

Method B

- Attach the electrodes and fill chamber as described above.
- Switch the HEADSTAGE SELECTOR switch to *on*.
- Switch the MODE SELECTOR switch to R MEM mode and read the series resistance from the meter.
- Set the FLUID RESISTANCE POTENTIOMETER to the value read from the meter in the last step. NOTE: While R MEM mode disables the FLUID RESISTANCE POTENTIOMETER, the setting will become active in all other operational modes.

Finally, see page 26 for a method of measuring fluid resistance with the epithelium in place (i.e. the fluid resistance adjustment need not be performed before the start of an experiment).

Analyzing the electrical properties of an epithelium

After mounting the epithelium into a chamber, connecting the current/voltage leads to the amplifier, and eliminating asymmetries in the voltage measuring electrode three basic properties of the epithelium can be measured. These are the transepithelial voltage, the transepithelial resistance and the short circuit current (I_{sc}; a measure of the net active ion transport across the epithelium).

Transepithelial voltage

The first measurement is straightforward and is simply read from the panel meter or from a chart recorder or oscilloscope. One must remember to make sure that there is no asymmetry potential between the voltage measuring electrodes. Since the transepithelial

voltage is measured *differentially*, one must know which one of the two voltage measuring electrodes is considered *zero* (or ground).

Transepithelial resistance

The transepithelial resistance can be measured by either passing a current across the epithelium (ΔI) and measuring the resultant voltage change (ΔV under current clamp mode) or by clamping the epithelium to a new voltage (ΔV) and measuring the change in current (ΔI under voltage clamp mode). The resistance is then calculated using Ohm's law which simply states that the resistance is equal to the change in the transepithelial voltage divided by the change in the transepithelial current,

$$R_{meas} = (\frac{\Delta V_t}{\Delta I_t}) A ,$$

where A is the area of the epithelium and the units of resistance are in ohms/cm^2. Since there is a finite distance between the voltage measuring electrodes and the epithelium, the calculated resistance (R_{meas}) is the sum of the transepithelial resistance (R_t) and the series resistance of the solution (R_s) (i.e. the resistance of the bathing solution between the tissue and each of the voltage measuring electrodes). This series resistance must be subtracted from R_{meas}. To determine the actual transepithelial resistance (R_t=R_{meas}-R_s), the **EC-825** can automatically subtract the series resistance (once it has been measured) from R_{meas}.

Series resistance is usually determined by measuring the resistance of the chambers in the absence of an epithelium. Since solutions of different ionic composition have different resistivities, R_s must be uniquely determined for each solution used. Although for high resistance epithelia, series resistance is only a minor correction (e.g. 1-2% of R_{meas}) for low resistance epithelia it can be 50% or greater of R_{meas}. An alternative approach for measuring R_s is offered on page 26.

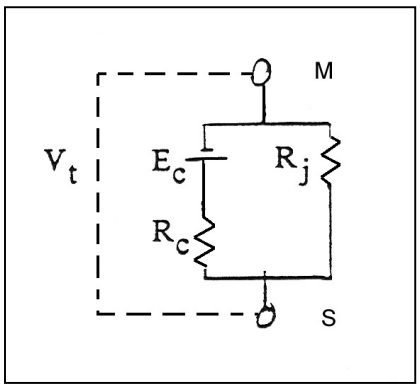
Short circuit current

The measurement of the short circuit current (I_{sc}) is deceptively simple. I_{sc} is simply defined as the current that must be passed across the epithelium to reduce the transepithelial voltage to zero. It is the current that short circuits the tissue. I_{sc} is measured by voltage clamping the epithelium to 0 mV and reading the applied current from the panel meter. When performing such measurements, it is essential that one compensates for the series resistance (R_s) by using the FLUID RESISTANCE control block on the **EC-825**.

Equivalent circuit analysis

We now introduced the concept that an epithelium can be modeled as an electrical circuit composed (in the most simple case) of a resistor and a voltage source. The justification for this electrical approach is that many epithelia produce a spontaneous potential even when bathed on both sides with identical solutions. Therefore, this potential can be represented as a voltage source. Since an epithelium is capable of restricting (resisting) the movement of ions between two compartments, it has resistive properties. Therefore, it can be

represented as a resistor. Below is the most simple equivalent circuit of an epithelium based on its morphology.



In the equivalent circuit shown to the right, the cells of the epithelium are represented by a resistor (R_c) in series with a voltage source (E_c), while the parallel tight junctions are represented by a simple resistor. This voltage source (also called the cellular electromotive force, EMF) is a complex function of the conductive properties of the cell membranes and the composition of the ions present in the bathing solution as well as in the cell interior. No voltage source is present in the junction since the tissue is bathed by symmetric solutions. For simplicity, we have left out a series (solution) resistor. Since parallel conductors add, the transepithelial conductance (G_t, the conductance between M, the mucosal solution and S the serosal solution) is given by

$$G_t = G_c + G_j .$$

Since resistance is the inverse of conductance ($G_t=1/R_t$), then the transepithelial resistance is given by

$$R_t = \frac{R_c R_j}{R_c + R_j}$$

and from Ohm's law, the current flow (i) around this circuit is

$$i = \frac{E_c}{R_c + R_j} .$$

Again using Ohm's law we find that the voltage difference across the epithelium (V_m-V_s=V_t) is equal to the current flow through the tight junction,

$$V_t = iR_j = \frac{E_c R_j}{R_c + R_j} ,$$

or through the cellular pathway,

$$V_t = E_c - iR_c = E_c(1 - \frac{R_c}{R_c + R_j}) = \frac{E_c R_j}{R_c + R_j} = \frac{E_c R_t}{R_c} .$$

Inspection of the above equations reveals three interesting features:

- The magnitude of the transepithelial potential is a function of both the cell and junction resistance, and the magnitude of the cell voltage source.

- The term (E_c/R_c) is equal to the short circuit current and is indeed equal to the current generating capability of the cells.
- At a constant E_c, a decrease in R_c will result in an increase in V_t. Thus as R_c approaches zero, V_t approaches E_c. Similarly, as R_c increases towards infinity, then R_t approaches R_j.

This last relationship can be derived and formalized in the following manner:

$$V_t = \frac{E_c R_t}{R_c}, \quad \textit{rearranging yields} \quad \frac{V_t}{E_c} = \frac{R_t}{R_c}$$

and

$$\frac{1}{R_t} = \frac{1}{R_c} + \frac{1}{R_j}, \quad \textit{rearranging yields} \quad 1 = \frac{R_t}{R_c} + \frac{R_t}{R_j}$$

substituting the above two equations yields

$$\frac{V_t}{E_c} + \frac{R_t}{R_j} = 1.$$

If a perturbation which changes only the cell resistance is performed, a plot of the resulting paired values of V_t and R_t (during this perturbation) yields a linear double intercept in which both R_j and E_c can be determined (*see* Figure 3). An important question is how does one know that the perturbation has altered only the cell resistance, and not the junction resistance R_j or the cell voltage source E_c? The best indicator will be that the plot is linear since a curvilinear plot suggests that the assumption of constant R_j and E_c has been violated.

A similar relationship can be derived if the experiments are performed under short circuit conditions. The derivation is shown below.

Recall that

$$G_t = G_c + G_j$$

$$\text{and } I_{sc} = E_c G_c \text{ or } G_c = \frac{I_{sc}}{E_c}.$$

Substituting gives

$$G_t = \frac{I_{sc}}{E_c} + G_j.$$

Using this equation, a plot of the transepithelial conductance (G_t) versus the measured short circuit current (I_{sc}) will have an intercept equal to the junctional conductance (the inverse of the junctional resistance) and a slope equal to the inverse cell voltage (i.e., $1/E_c$).

This equation has the same assumption as the previous plotted equation, (i.e. the experimental perturbation only changes the cellular resistance or conductance).

Experience has shown that a plot of V_t *vs.* R_t is more sensitive to changes in either R_j or E_c than a plot of G_t *vs.* I_{sc}. The reason for this is that whereas I_{sc} is a measure of only the cellular pathway (it is not affected by the junctional resistance), V_t is a function of both the cellular pathway and the junctional resistance and is thus more sensitive to a change in either parameter. This is illustrated below (Figure 3) in which the experimental conditions were such that both R_c and E_c are changing. Note that although the G_t *vs.* I_{sc} plot is linear, the plot of V_t *vs.* R_t is non-linear.

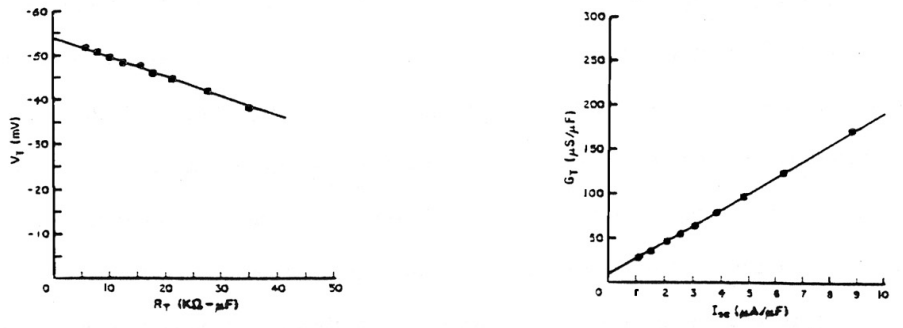


Figure 2. Plot of V_t versus R_t (left panel) and G_t versus I_{sc} (right panel). In this example the cell membrane resistance (of the rabbit urinary bladder epithelium) was decreased using the pore forming antibiotic gramicidin D. Of importance is that the mucosal solution was designed to mimic the ionic composition of the cell interior and as a consequence increasing the apical membrane resistance will not alter the cell EMF. This is confirmed since both plots are linear and yield near identical values for E_c and R_j.

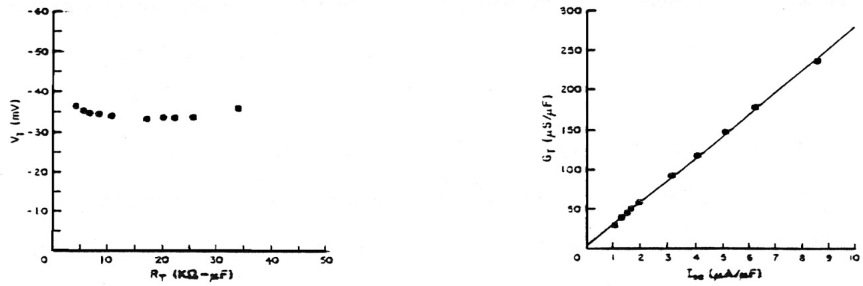
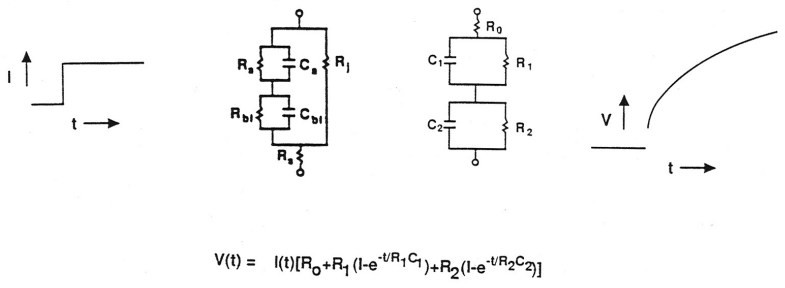


Figure 3. A plot of V_t *vs.* R_t (left panel) and G_t *vs.* I_{sc} (right panel). In this example the cell membrane resistance was decreased using gramicidin D. In addition the mucosal solution was selected such that the cell EMF (E_c) changes during gramicidin action on the cell resistance. This is illustrated by the fact that the plot of V_t *vs.* R_t is non-linear, and indicates that one of the assumptions of the equation has been violated (in this instance E_c is not constant). Note, however, that the plot of G_t versus I_{sc} is reasonably linear even though the assumption of a constant E_c has been violated. To use this method only the resistive (conductive) properties of the cellular pathway must change and the change must be sufficiently large to result in a significant change in the measured parameters, (i.e. V_t, R_t, I_{sc} or G_t). As a consequence, this method is most profitably used on the so called tight epithelia, since a change in the cellular resistance results in a significant change in R_t. In leaky epithelia, where R_j can be 10 fold lower than R_c, large perturbations (in this case a decrease) in R_c must be produced to obtain a reliable (measurable) decrease in R_t.

Three approaches have been used to alter R_c in a controlled manner. These are increasing the cell membrane conductance to a given ion using second messenger systems, decreasing the conductance to a given ion using pharmacological blockers, and lastly, artificially increasing the membrane conductance using pore forming agents such as gramicidin D, nystatin or amphotericin B. When using pore forming agents, one must use a mucosa solution which mimics the cell interior (i.e. the potassium content must be high while the calcium, sodium and chloride content must be low). Typically, one performs an equimolar replacement of sodium with potassium and chloride with a large monovalent anion such as gluconate. Such a solution exchange has two advantages. First, since there is low chloride in the bathing solution, cell swelling due to KCl influx is minimized, and secondly, since the mucosal and cell ion concentrations are matched, there will be no change in the apical membrane voltage when the pore forming agent is added (i.e. E_c will not be affected as R_c is decreased). Additionally, since the ion concentrations are matched, the value of E_c will be approximately equal to the value of the voltage source of the basolateral membrane.

Determining series resistance and epithelial surface area

In this section we discuss how the series (fluid) resistance can be estimated the with the epithelium in the chamber, as well as how to estimate the surface area of the epithelium. First, we must draw an equivalent electric circuit which describes the epithelium and the fluid resistance in terms of its resistive and capacitative properties. In this circuit, the fluid resistance and the tight junction are modeled as resistors while the apical and basolateral membranes of the epithelium are represented by a parallel arrangement of a resistor and capacitor. The figure below shows this circuit (the tight junctional resistance is not included since it is infinite) and outlines the response of the circuit to a square current pulse. The characteristic equation describing the voltage response to the current input signal is also shown. Note that the voltage response to a square current waveform is time-dependent. Consequently, this response can be analyzed in the time domain.



$$V(t) = I(t)[R_0+R_1(1-e^{-t/R_1C_1})+R_2(1-e^{-t/R_2C_2})]$$

Figure 4. Response of a simple epithelial equivalent circuit to a square current input, and the generalized equation which describes the voltage output. The output response is shown as two easily discernible exponential traces. In this example, the value of the two time constants (R_1C_1 and R_2C_2) were selected to be greatly different. If the time constants are approximately equal, this circuit will show a single exponential response similar to the single R_c with series resistor. The voltage (V), current (i) and time (t) scales are in arbitrary units. This figure demonstrates the response through a series resistor and two parallel resistor capacitor combinations arranged in series. This response is characterized by an initial voltage jump (equal to the fluid resistance) followed by the sum of two hyperbolic curves, each curve being a function of an individual resistor/capacitor network.

MEASURING FLUID RESISTANCE IN THE PRESENCE OF AN EPITHLIAL MEMBRANE

The magnitude of the voltage jump shown in Figure 4 divided by the magnitude of the current step will be equal to the fluid resistance. A square current pulse (the rise time of the current step must be less than 10 μs) must be used to perform this measurement.

The following protocol can be used to make this measurement:

- Set the MODE SELECTOR switch to *c clamp*.
- Set the CLAMP RESPONSE to *fast*.
- Connect the V MONITOR BNC to a high speed oscilloscope and make sure that the voltage signal is not being filtered by the oscilloscope amplifier.
- Apply a square current step to the EXT CLAMP COMMAND input BNC.
- Measure the magnitude of the initial voltage jump. Since the V MONITOR output BNC has x10 gain, divide the measured voltage by 10.
- Divide the measured voltage by the magnitude of the applied current step. The resulting resistance is equal to the fluid resistance.

MEASUREING EPITHELIAL SURFACE AREA

To estimate the epithelial surface area one must analyze the time dependent change in the membrane voltage. The complete protocol for analyzing these voltage transients has been described in detail by Lewis and Demoura (1984). In brief, the "on" voltage response to a square current pulse is digitized (at 100 μs/point with a resolution of 0.05 mV) and stored in computer memory. This data is next converted to an "off" voltage response by subtracting the time-dependent "on" voltage response from the pre-stimulus voltage. The absolute value of this voltage change ($|\Delta V|$) is stored together with the corresponding time (t; where t=0 is the time at which the current was applied). $|\Delta V|$ is then fit to a sum of exponentials using standard non-linear curve fitting routines.

The number of exponentials used is limited by the equivalent circuit. In general, analysis will yield two voltage terms, which are converted to resistances by dividing the voltage by the amplitude of the current pulse, and two time constants (τ), each of which is the product of a resistor and capacitor. The relationship between the best fit values and the actual epithelial resistors and capacitors depend upon the equivalent circuit model used to represent the epithelium. This is a problem of the non-uniqueness of these equivalent circuits.

FURTHER CONSIDERATIONS

Let us now consider the basic epithelial equivalent circuit model. In Figure 4 we show two circuits. The general form of the equations describing the voltage response of these circuit to a square current pulse are identical. The relationship between the resistors and capacitors in these circuits is described by the following equations (*see* Lewis and Demoura, 1984):

$$\frac{C_1 C_2}{C_1 + C_2} = \frac{C_a C_{bl}}{C_a + C_{bl}}$$

$$\frac{R_1 + R_2}{R_1 C_1 R_2 C_2} = \frac{R_a + R_{bl}}{R_a C_a R_{bl} C_{bl}}$$

$$\frac{1}{R_1 C_1} + \frac{1}{R_2 C_2} = \frac{R_j + R_a}{R_j R_a C_a} + \frac{R_j + R_{bl}}{R_j R_{bl} C_{bl}}$$

$$\frac{1}{R_1 C_1 R_2 C_2} = \frac{R_j + R_a + R_{bl}}{R_j R_a C_a R_{bl} C_{bl}} .$$

Note that a five parameter model (for simplicity we ignore the solution resistance R_s) can be determined by 4 parameters. To curve fit this five parameter model to the impedance data, we must have an independent estimate of one parameter. For example, Clausen *et al.* (1979) measured the ratio of the apical to the basolateral membrane resistances for the rabbit urinary bladder, while Wills and Clausen (1985) independently measured the resistance of the tight junctions using either antibiotics or the sodium channel blocker amiloride.

Of interest is that the first equation demonstrates that the product of the capacitors divided by the sum is independent of the equivalent circuit model. This value is called the effective capacitance and since in most epithelia, C_{bl} is greater than C_a (by about a factor of 5), the effective capacitance is approximately equal to C_a. This relationship has been used to measure the change in membrane surface area as a function of alterations in epithelial transport rate.

APPENDIX

References and recommended reading

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Lewis, S.A. and Hanrahan, J.W. 1989. *Methods in Enzymology* **192:** 632-650

Lewis, S.A. and. deMoura, J.L.C. 1984. *Journal of Membrane Biology* **82:**123-136.

Ussing, H.H. and Zerahn, K. 1951. *Acta Physiologica Scandinavica* **23:**111-127

Wills, N.K. and Clausen, C. 1987. *Journal of Membrane Biology*. **95:**21-35.

Methods in Enzymology Volume 192. Biomembranes Part W. Cellular and Subcellular Transport Epithelial Cells. 1990. An excellent book that gives detailed methods used to study a variety of epithelia. Includes a discussion on such topics as edge damage and solution resistance.

Methods in Enzymology Volume 171. Biomembranes Part R. Transport theory: Cells and Model Membranes. 1989. Similar to the above book. Includes good chapters on solution resistance, edge damage artifacts, epithelial impedance, liquid junction potential etc.

Chloriding silver wires

Before using Ag wires as current electrodes, they must be chlorided. New (previously unused) wire should first be cleaned with ETOH before continuing, while previously chlorided wire should have the old chloride coating removed. Two methods are commonly used to chloride Ag wire; soaking a clean wire in household bleach or electroplating a clean wire using a voltage source. Both methods are described below.

A) Soaking in bleach - Simply immerse the wire in full strength common household bleach (Clorox) for 15 to 30 minutes until a purple-gray color is observed. Rinse and use.

B) Electroplating - Electroplating a silver wire with chloride is achieved by making the wire positive with respect to a solution containing NaCl (0.9%) or KCl (3M) and passing a current through the electrode at a rate of 1 mA/cm^2 of surface area for 10-15 seconds or until adequately plated (a 1 cm length of 1 mm diameter wire will require approximately 0.3 mA). The color of a well plated wire should be purple-gray. Periodic reversal of the polarity while plating the electrode tends to yield a more stable electrode.

When electroplating a previously plated wire, you may find that it does not plate evenly. Complete removal of the residual silver chloride is usually necessary to effect a uniform coat. Before making the wire positive to the chloriding solution, reverse the polarity for 5 to 10 seconds to remove any remaining chloride that might be left in pits on the wire. Then proceed as described above.

Adjusting the fluid resistance measurement range

Unless otherwise specified, the FLUID RESISTANCE measurement range is factory set for 0-100 Ω. If required, the FLUID RESISTANCE range can be extended to 1 kΩ by setting jumpers on the main circuit board. Each channel must be adjusted separately.

The procedure to make this adjustment is as follows:

- Remove the five Philips-head screws holding the top cover in place and remove the cover.
- Locate the jumper blocks labeled W1-W8. W1-W4 are located on a single block next to the piggy-backed circuit board. W5-W6 and W7-W8 are located on two jumper blocks located towards the front of the main circuit board.
- To set the channel for 1 kΩ operation unjumper W1, W3, W5, and W7 and jumper W2, W4, W6, and W8.
- To set the channel for 100 Ω operation jumper W1, W3, W5, and W7 and unjumper W2, W4, W6, and W8.

Specifications

Headstage

Input Impedance:
1 x 10^{10} Ω shunted by 10 pF

Input Voltage: ±1.5 V maximum

Common Mode Voltage: ±13 V maximum

Common Mode Rejection: 120 dB @ 60 Hz

Leakage Current: 500 pA max.

Offset Voltage Range: ±120 mV

Voltage Clamp

Ranges:

Internal Clamp Potentiometer:
± 100 mV with 10-turn control

External Command: ±1 V

External Command factor:
1 mV/10 mV applied

Current Clamp

Ranges:

Clamp Potentiometer: ±1 mA

External Command: ±10 mA

Command Factor: 1 µA/10 mV applied

Speed: 10 µs measured with model membrane

Compliance

EC-825: ±120 V

EC-825LV: ±15 V

Resistance

Fluid resistance Compensation range:

0-100 Ω standard

0-1 kΩ optional

Membrane Resistance Measurement: made with a 2 Hz bipolar constant current square wave

Ranges:

0-2 kΩ, injected current = 10 µA

0-200 kΩ, injected current = 1 µA

Panel Meter: 3.5 digit LCD

Voltage Range: 200 mV maximum

Current Range: 2000 µA maximum

Timers (A & B)

Range:
10 ms to 1000 seconds, set with 2 digit resolution and 4 ranges (each channel)

Power Requirements

100-130 VAC or 220-240 VAC, 50/60 Hz

Physical Dimensions

EC-825 and EC-825LV:
17.8 cm H x 42 cm W x 25 cm D

Headstage: 7.7 cm H x 7.7 cm W x 5 cm D

Shipping Weight

EC-825 and EC-825LV: 9.1 kg

Warrantee:

Three years, parts and labor