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OPERATIONS MANUAL

MODEL VCC600 REVISION G
SINGLE CHANNEL VOLTAGE-CURRENT CLAMP

SERIAL NO. _____

PHYSIOLOGIC INSTRUMENTS, INC.

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San Diego, CA 92128
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NOTICE: The Voltage-Current Clamp Mainframe and Headstage are matched units and have the same serial number. Mainframes and headstages may be interchanged, however recalibration of the instrument may be required. It is suggested that headstage units be interchanged only for trouble-shooting purposes

WARRANTY: This instrument is warranted against defective materials and workmanship for one year from date shipped. All requests for repair or replacement parts should be directed to the above mailing address.

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PHYSIOLOGIC INSTRUMENTS

VOLTAGE-CURRENT CLAMP
MODEL VCC600

INSTRUMENT DESCRIPTION: The model VCC600 voltage/current clamp has been designed to be a flexible tool to aid in the study of ion transport mechanisms by *in vitro* epithelial tissues. The VCC600 provides the capability to clamp either the voltage or the current across the epithelium and is ideally suited for both electrophysiological studies as well as ion flux studies in a number of epithelia. The model VCC600 offers features such as:

1) a built-in pulse generator that allows continuous monitoring of the tissue conductance;
2) digital readout of the transepithelial voltage or current;
3) compensation for electrode asymmetry potentials;
4) automatic compensation for voltage errors due to the resistance of the fluid in series with the epithelium - fluid resistance compensation;
5) the ability to clamp either the voltage or current over the range ±300 mV or μA;
6) recorder outputs for continuous monitoring of the transepithelial voltage and current;
7) a pulse generator output;
8) an external command signal input to allow an arbitrary waveform to be clamped;

and,

9) a remote control interface jack to allow the VCC600 to be controlled from a remote device such as a computer.

DESCRIPTION OF CONTROLS

The front panel controls of the VCC600 are divided into six sections - clamping circuitry, meter, power, pulse generator, input/output connection and balance adjustment.

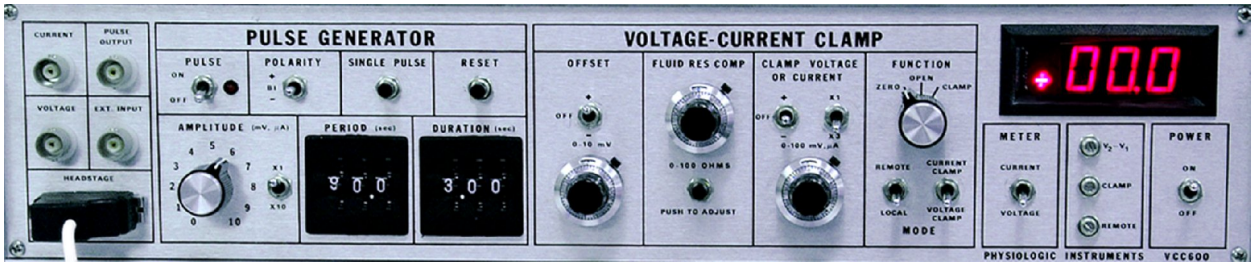
I. CLAMPING CIRCUITRY

OFFSET - range ±10 mV. Adjusted by means of a 10-turn calibrated potentiometer. Used to correct for any voltage asymmetry between the voltage-sensing electrodes.

FLUID RESISTANCE COMPENSATION - range 0-100 Ω. Adjusted by means of a 10-turn calibrated potentiometer. A test current may be injected by means of a pushbutton switch when the FUNCTION switch (see below) is in the ZERO or OPEN position.

CLAMP VOLTAGE OR CURRENT - determines the D.C. level at which the voltage or current will be clamped. Polarity and range are switch selectable while level is set by means of a 10-turn calibrated potentiometer. Range: ±100 and ±300 mV or μA.

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FUNCTION - selects whether: 1) the inputs of the voltage sensing amplifiers are to be internally grounded (ZERO); 2) the feedback circuitry is to open circuited (OPEN) so that the epithelium is in the "open circuit" condition; or, 3) the feedback loop is completed (CLAMP) so that the tissue is either voltage clamped or current clamped at the level given by the CLAMP VOLTAGE OR CURRENT potentiometer.

MODE - consists of two sections

1) VOLTAGE/CURRENT - selects whether the transepithelial voltage or the transepithelial current is to be clamped when the **FUNCTION** switch is set to **CLAMP**.

2) LOCAL/REMOTE -determines whether the selection of clamp FUNCTION, VOLTAGE/CURRENT MODE, and CLAMP VOLTAGE OR CURRENT level are to be controlled using the front panel controls (LOCAL) or by external device such as a computer (REMOTE).

II. METER

Selects either the transepithelial voltage (V_2-V_1) or current (the polarity is normally positive for current flowing from side 1 to side 2 across the tissue) to be displayed on the digital panel meter. The meter may be read directly in mV or µA over the range ±199.9 mV or µA. (Note: both the polarity and the gain of the current measuring amplifiers may be altered using internal jumpers. See Internal Jumper Selection below.)

III. POWER

Turns device ON or OFF

IV. PULSE GENERATOR

PULSE (ON - OFF) - turns pulse on or off to the clamp circuitry and to the PULSE OUTPUT connector.

POLARITY - selects polarity of unipolar pulses or selects bipolar pulses (center position on switch)

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RESET - resets the period timer to zero and elicits a pulse.

SINGLE PULSE - injects a single pulse without interrupting the period timer.

PERIOD - allows digital selection of the interpulse interval in seconds. The range is typically 0-99.9 sec.

DURATION - allows digital selection of the duration of the pulse in seconds. The range is 0-9.99 sec. Restriction: the duration may not exceed one-half the period or timing errors will result.

AMPLITUDE - Allows selection of pulse amplitude in steps of 1 mV (or μA) over the range 0-10 mV (μA) or in steps of 10 mV (μA) between 0 and 100 mV (μA) when used with the clamp circuitry. Pulse amplitude at the PULSE OUTPUT connector will be 10x this value (mV only).

V. INPUT/OUTPUT CONNECTIONS

CURRENT - allows continuous monitoring of the transepithelial current. Output is factory set to 10mV/μA but is jumper selectable (see Table 1). Current output is positive for current flow from 1 to 2.

VOLTAGE - allows monitoring of the transepithelial voltage (V_2-V_1). Output is at x10 gain - i.e., 10mV/mV.

HEADSTAGE - connection to HEADSTAGE unit.

EXTERNAL INPUT - allows any external waveform to be clamped. Signal is reduced 10-fold so that a 100 mV input signal will cause the tissue to be clamped at 10 mV or μA depending on the clamp MODE.

PULSE OUTPUT - allows monitoring or use of the pulse generator output when the PULSE ON/OFF switch is in the ON position. Output is at x10 gain. (Note: By connecting the PULSE OUTPUT to the EXT. INPUT the range of the PULSE AMPLITUDE is exactly doubled - e.g. ±200 mV in 20 mV increments.)

REMOTE - See Rear Panel connectors below.

VI. BALANCE ADJUSTMENT - Permits easy adjustment of critical amplifier balance potentiometers from the front panel. See Calibration adjustments below.

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HEADSTAGE DESCRIPTION: The HEADSTAGE is an active unit which contains the voltage sensing amplifiers and a current-to-voltage converter which provides the "ground" to the I_1 side of the epithelium.

> **NOTE: V_1 and I_1 must be connected to the same side of the epithelium. Similarly for V_2 and I_2.**

A unity gain buffer amplifier in the HEADSTAGE permits the voltage at either V_1 or V_2, selected by means of pushing the lever of the miniature toggle switch located between the V_1 and V_2 input jacks toward the desired voltage input, to be accessed via the RED binding post on the rear panel. This signal may be used to drive the circuit ground on an electrometer in order to record the intracellular potential referenced to either V_1 or V_2. Alternatively, it may be used in pH stat experiments to drive the low, or reference, input to a pH meter.

REAR PANEL CONNECTORS

RED	-	Headstage buffer amplifier output. See above.
BLACK	-	Circuit ground.
GREEN	-	Chassis ground.
REMOTE	-	Allows the clamping circuitry to be controlled by an external device. See below.
POWER	-	Power input module for connecting the MAINS power and for selecting the appropriate mains voltage (110 or 220 volts, 60 or 50 Hz).

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GENERAL OPERATING INSTRUCTIONS

INITIAL SETUP: Set all front panel switches to OFF, the FUNCTION switch to ZERO, and the MODE switches to LOCAL, VOLTAGE. All calibrated potentiometers should be set to 0.0 (i.e., turned fully counterclockwise). Insert the headstage 9 pin male DE9 connector into the HEADSTAGE socket on the front panel and secure by sliding the locking mechanism to the left. Select the appropriate mains voltage using the switch on the power inlet module on the rear panel and attach the AC cord provided.

IMPORTANT: IF THE POWER INPUT SELECTOR IS NOT CORRECTLY SET TO THE MAINS VOLTAGE, *SEVERE DAMAGE* TO THE INSTRUMENT WILL RESULT.

TYPICAL EXPERIMENT

Fill an "Ussing" type chamber with physiological saline and connect appropriate electrodes to inputs V_1 and V_2 for measuring of the transepithelial voltage. Connect current passing electrodes to I_1 and I_2 ensuring that V_1 and I_1 are connected to the same side of the epithelium. Turn the **POWER** switch to **ON** and allow 5-minutes for instrument warm-up. The digital panel meter should read zero when the **METER** switch is in either the **VOLTAGE** or **CURRENT** position. Switch the **FUNCTION** switch to **OPEN** and the **METER** switch to **VOLTAGE**. The voltage (V_2 - V_1) is the asymmetry voltage between the voltage measuring electrodes and is the sum of asymmetry in the electrodes themselves and all liquid junction potentials in the voltage measuring circuit. To compensate for these place the **OFFSET** switch in either the plus or minus position and adjust the **OFFSET** control so that the voltage reads 0.0 on the panel meter. If the reading on the meter is not stable, is off scale (i.e., > ±199.9 mV), or cannot be compensated, the problem usually involves an air bubble or discontinuity in one of the voltage sensing limbs of the circuit. When the electrode **OFFSET** is properly adjusted place the **METER** switch on **CURRENT** and press and hold the **FLUID RESISTANCE COMPENSATION** test button. Verify that a current of approximately 60-68 μA is registered on the meter. [NOTE: A value less than this indicates that the resistance of the current passing bridges or electrodes is excessive. While this in itself will not prevent the clamp from working, it does limit the amount of current that can be passed and is poor experimental design in that it increases errors due to non-ideal rejection of the common mode voltage resulting from current flow between the chamber and the I_1 input to the HEADSTAGE. It is recommended, therefore, that the resistance of the current passing bridges be kept low by making them as short as possible and/or by increasing their cross-sectional area.] While still passing current, switch the **METER** switch to **VOLTAGE** and adjust the **FLUID RES COMP** control so that V_2-V_1 on the panel meter again reads 0.0. At this point the value of the resistance due to the fluid in series with the tissue may be read from the calibrated dial and will be compensated for automatically at any current - i.e., the actual voltage across the epithelium will be determined and will appear on the digital panel meter and at the **VOLTAGE OUTPUT BNC** connector (x10). In the event that a current cannot be passed when the test button is pushed, the problem usually involves an air bubble in one of the current passing agar bridges. Now place the **FUNCTION** switch back on **ZERO** and mount a tissue in the chamber being careful not to change the distance between the tips of the voltage

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sensing electrodes. Place the **FUNCTION** switch on **OPEN** and the **METER** switch on **VOLTAGE** to read the open circuit voltage generated by the tissue (already corrected for electrode asymmetry potentials). Place the **FUNCTION** switch on **CLAMP** with the **MODE** switch on **VOLTAGE** and verify that (V_2 - V_1) now reads 0.0 - i.e., the tissue is short-circuited. Place the **METER** switch to **CURRENT** to read the short circuit current, Isc. The transepithelial voltage may now be clamped to virtually any voltage using the **CLAMP VOLTAGE or CURRENT** controls and/or the **PULSE GENERATOR** circuitry.

REMOTE CONTROL

When the **MODE** switch is placed on **REMOTE** the front panel **FUNCTION**, **MODE - VOLT/CURRENT**, and **CLAMP VOLTAGE or CURRENT** controls are disabled. All other controls are unaffected. Control of these disabled features must be supplied via the **REMOTE** interface on the rear panel. Details of this feature are given below.

REMOTE INTERFACE CONNECTIONS:

On the rear panel is a 9 pin female DE9 subminiature connector by which interface may be made to an external controller such as a computer or a simple battery and switch. The pin connections are:

PIN #	Description
1	Selects CURRENT or VOLTAGE MODE; this must be a TTL compatible logic signal. Logic high (+3.5 - 5 V) selects CURRENT, logic low (0 - 0.8 V) selects VOLTAGE.
2	Analog signal input high; this signal replaces the CLAMP VOLTAGE or CURRENT signal and may be the output of a D/A converter, etc.
3	Measured transepithelial voltage (V_2 - V_1) at 10 mV/mV (same signal as at the VOLTAGE BNC connector).
4	Circuit or signal ground. (Note: This should not be connected to the signal ground on the computer or unwanted noise may result.)
5	Not connected.
6	Selects FUNCTION - OPEN or CLAMP; logic high (+3.5 - 5 V) selects CLAMP; logic low (0 - 0.8 V) selects OPEN. (Note: front panel FUNCTION switch must be in either OPEN or CLAMP position.)
7	Analog signal input low; this signal may be the analog ground of the D/A converter which typically should be isolated from the system ground.
8	Measured transepithelial current (same as at the CURRENT BNC connector).
9	Shield or Chassis ground.

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INTERNAL JUMPER CONFIGURATIONS

Unless otherwise specified upon ordering, the VCC600 is factory configured as follows:

DESCRIPTION	VALUES	JUMPER POSITION
CURRENT measurement: output at BNC:	10 mV/µA	J1-A, J2-A
range displayed on meter:	±199.9 µA	J4-C
polarity:	(+) for cation flow from side 1 (I_1) to side 2 (I_2).	J2-D
range at BNC:	±1.3 mA (±13 V)	J1-B, J2-A
FLUID RESISTANCE COMPENSATION RANGE:	0 - 100 Ω	J3-A
MAXIMUM I_2 OUTPUT VOLTAGE:	±13 V	J5-B,C
(option HV only)	±35 V	J5-A,D

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The function and settings for each jumper are described below:

TABLE of JUMPER SETTINGS

JUMPER	LOCATION	FUNCTION or SETTING
J1	(on HEADSTAGE pc board)	Sets the voltage output gain of the current measuring amplifier. A - 10 mV/µA B - 1 mV/µA
J2	(above U21)	Inverts measured current and multiplies by: A - x1 B - x100 Polarity of current displayed: C - (-) for cation flow from 1 to 2 D - (+) for cation flow from 1 to 2
J3	(to right of U23)	Sets fluid resistance compensation range. The range is given by the product of the current amplification determined by J1 and J2 and the factor selected below: A - x0.01 B - x0.02 C - x0.05 D - x0.10 For example: J1-B, J2-B, J3-C yields a resistance compensation range of: J1-A J2-B J3-C 1 mV/µA * 100 * 0.05 = 5000 Ω
J4	(to right of U18)	Sets the decimal point location for the displayed current only. Proper setting of this jumper depends on the selections made for J1 and J2. A - ±1.999 B - ±19.99 C - ±199.9 D - ±1999
J5	(to right of U19)	Selects power supply voltage to the clamp amplifier (U18). Settings must be made in pairs - e.g., B & C or A & D. A - -35 V (option HV only) B - -15 V C - +15 V D - +35 V (option HV only)
J1a	(on Main circuit board at right of U14)	J1a-A normal. J1a-B reverses the polarity of the pulse generator output. In bipolar mode it reverses the order of the sequential pulses.

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CALIBRATION

EQUIPMENT NEEDED:

1. Dummy membrane with internal voltage source (model DM660 or equivalent, Physiologic Instruments, 12335-6 World Trade Dr, San Diego, CA 92128).
2. Digital voltmeter (DVM) having sensitivity of 100 µV.
3. Oscilloscope with vertical input sensitivity of at least 2 mV/div.

POWER SUPPLY CALIBRATION:

(Note: Because of the potential for electrical shock, calibration of the power supply section should be undertaken only by qualified personnel.)

1. **UNPLUG the instrument!**
2. Remove the four Phillip's oval head screws from the top panel of the instrument and slide the cover backwards to allow access to the interior. DON'T LOSE THE SCREWS - THEY ARE METRIC (M3).
3. Unplug the card-edge connector from the digital panel meter on the right side of the instrument and remove the two screws from the rear cover plate on the aluminum box beneath the panel meter. Do not remove the meter.
4. As a precautionary measure, examine the line voltage selector switch on the power inlet module on the rear panel and ensure that it is correctly set for the mains voltage to be used (110 V or 220 V).
5. Plug the instrument into the mains line and turn the POWER switch to ON.
6. Connect the negative input of the DVM to the circuit ground (black banana jack on rear panel) and the positive input to -15V on the power input connector. Be very careful not to short the + and - supply voltages to each other. Adjust R1 on the power supply circuit board so that the output is -15.00 V. Move probe to +15 test point and adjust R2 to register +15.00 V on the DVM. If option HV is present, calibrate the -35 and +35 V outputs using R5 and R6, respectively. Take care not to contact the fuse holder during this procedure.
7. **UNPLUG** the instrument and return the POWER switch to OFF.
8. Replace the cover plates and screws on the power supply chassis.
9. Reconnect the meter edge-card connector.

INSTRUMENT CALIBRATION:

Plug the Dummy membrane into the HEADSTAGE, set the front panel switches as indicated below, turn the POWER switch to ON and allow 5 minutes for the instrument to warm up.

FUNCTION	- ZERO
MODE	- LOCAL, VOLTAGE CLAMP
CLAMP VOLTAGE or CURRENT	- 0.0, OFF, x1
FLUID RES COMP	- 0.0
OFFSET	- 0.0, OFF
PULSE	- OFF
AMPLITUDE	- 10, x1

A. REMOTE buffer amplifier balance:

1) Attach reference probe from the oscilloscope to the shield of any of the BNC connectors on the front panel or to the analog ground output banana jack (black) on the rear panel.

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2) Attach the test probe from the oscilloscope to TP5 (test point 5) and set the vertical sensitivity at 1 mV/div.
3) Adjust the trimpot labeled REMOTE on the front panel for 0 volts output.

NOTE: This procedure simply puts the remote buffer amplifier into reasonable balance. When using a remote voltage source such as a computer (digital to analog converter) to drive the clamp this REMOTE balance pot may be used to nullify any offset voltage arising from the remote voltage source.

B. CURRENT balance:

1) Attach test probe to TP7.
2) Remove the cover from the HEADSTAGE unit.
3) Adjust the BALANCE trimpot (R3) on the HEADSTAGE PC board for zero output on the oscilloscope.
   If J2-D was selected the digital panel meter should now read 0.0 when the METER is switched to CURRENT.

C. Current inverter amplifier balance:

1) Attach test probe to TP6.
2) Adjust R61 for zero output.

D. CLAMP VOLTAGE OR CURRENT level balance:

1) Attach test probe to TP9.
2) Adjust R76 to read 0.0.
3) Flip the gain switch to x3 and back to x1. "Fine tune" the R76 so that no deviation in output is observed when the gain switch is changed.

E. VOLTAGE (V_2 - V_1) adjustments - balance:

1) Attach the test probe to TP8 or to the VOLTAGE output BNC connector on the front panel.
2) Balance - Adjust V_2 - V_1 trimpot on the front panel for zero output on the oscilloscope and 0.0 on the digital panel meter.

F. CLAMP amplifier balance:

1) Turn the dummy membrane ON (unshorted) and check to see that the appropriate voltage appears on the digital panel meter.
2) Confirm that the MODE switch is set to LOCAL, VOLTAGE CLAMP.
3) Turn the FUNCTION switch to CLAMP.
4) On the oscilloscope check to see that the clamp is not oscillating (wideband oscillation will illuminate the entire screen). If the clamp is oscillating, return the FUNCTION switch to OPEN and change the frequency response of the clamp amplifier by switching in more capacitative feedback using S8 on the main PC board. If the clamp does not oscillate, then proceed.
5) Adjust the CLAMP trimpot on the front panel so that the VOLTAGE, V_2 - V_1, reads 0.0 on the panel meter and oscilloscope. Switch the MODE switch to CURRENT CLAMP and the METER to CURRENT and check that 0.0 current is displayed. This value (and TP7) should not change when the FUNCTION switch is returned to the OPEN position.

G. OFFSET calibration:

1) Set: FUNCTION - ZERO
   METER - VOLTAGE

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OFFSET polarity - +
OFFSET pot - 6.00

2) Adjust R69 so that the digital panel meter displays 6.0 mV.
3) Flip the polarity switch to (-) and verify that the meter reads -6.0 mV.
4) Return the polarity switch to OFF.

H. CLAMP VOLTAGE OR CURRENT level calibration:

1) Set: FUNCTION - CLAMP
MODE - LOCAL, VOLTAGE CLAMP
METER - VOLTAGE
CLAMP LEVEL polarity - OFF
CLAMP gain - x1
CLAMP pot - 60.0
2) Check that the clamped voltage still reads 0.0.
3) Switch CLAMP polarity to (+).
4) Adjust R74 so that the digital panel meter displays 60.0 mV.
5) Flip the polarity switch to (-) and verify that the meter reads -60.0 mV.
6) Flip the CLAMP gain to x3 and see that the digital panel meter displays -180.0 ± 0.5 mV.
7) Return the polarity switch to OFF.

I. PULSE GENERATOR timing: Timing is controlled by a crystal clock. No adjustment is required.

J. PULSE GENERATOR amplifier balance:

1) Voltage clamp the dummy membrane to 0.0 mV.
2) Set: PERIOD - 90.0 sec
DURATION - 0.01 sec
AMPLITUDE - 10, x1
PULSE - ON
POLARITY - **BI**polar
METER - VOLTAGE
3) Adjust R33 so that the digital panel meter reads 0.0 mV.
4) Flip the AMPLITUDE gain switch to x10 and "fine tune" R33 so that no deviation is seen at TP8 when the PULSE ON/OFF switch is toggled back and forth.
5) Set: GAIN - x1
DURATION - 4.00 sec
Depress the PULSE RESET button momentarily and adjust R28 so that +10.0 and then -10.0 mV appears on the digital panel meter (since this pulse only lasts 4 sec it may be necessary to retrigger the pulse using either the RESET or the SINGLE PULSE buttons).
6) Flip the Gain switch to x10. Adjust R31 so the digital panel meter displays +100.0 and then -100.0 mV during the pulses.

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Optional **DM660 Dummy Membrane**

Description: The DM660 is a simple dummy test membrane for connection of single Ussing chamber setups to the VCC600 Voltage-Current clamp. It is powered by one AAA battery located under the bottom cover plate. It is activated when the toggle switch is placed on “Test” and turned off when the switch is on “Setup”. Leaving the switch on Test will cause the battery to run down over time.

Operation:

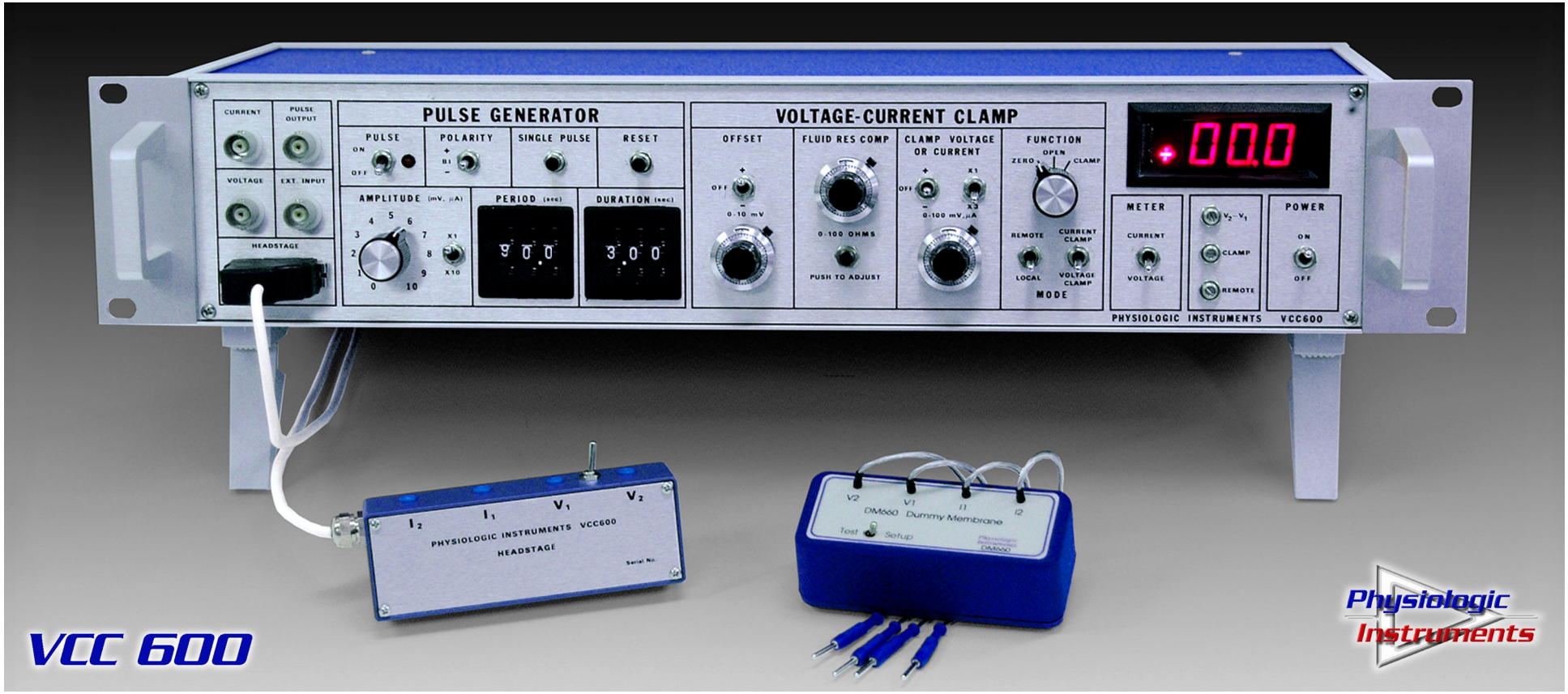
The dummy membrane may be used before or during an experiment to trouble-shoot problems and determine whether a problem resides in the electronics or the experimental setup. To use the dummy membrane push the toggle switch to "Setup". This position shorts the membrane resistance and is equivalent to setting up an Ussing chamber without a tissue. The offset adjustment on the clamp should be 0.0 mV when the Function switch is set to “Open”. Fluid resistance compensation should be adjusted under this condition and should be 50 ohms. Switching the toggle switch to "Test" connects the battery to the dummy membrane and is equivalent to mounting a tissue in the Ussing chamber An open circuit voltage of approximately 25-26.5 mV should appear on the meter (voltage position). Switching the function switch to “Clamp” (Mode switch on Voltage”) should cause the voltage to go to 0.0 and the short-circuit current to go from 0 to ~80-90 μA. When finished with the dummy membrane, the toggle switch should be pushed back to prevent run down of the battery.

Standard membrane version.
With new battery provides the following values:
Rm= 296 Ohm
Rfl = 50 Ohm
Vm~ 26.4 mV
Isc~ 89 uA

Special high membrane resistance membrane version.
Modified version with new battery provides the following values:
Rm= 3.3K resistance
Rfl = 604 Ohm
Vm~ 12.0 mV
Isc~ 3.1 uA

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