The Frogometer, an Instrument for Measuring and Clamping Voltages

By JOHN R. MENNINGER

A number of interesting experiments that college biology students can perform require the measurement of weak voltages of biological origin. I should like to describe an instrument that combines a sensitive millivolt meter of high input impedance with a voltage-clamping device. The latter feature allows students to measure membrane properties, such as the short-circuit current in the isolated frog skin (H. H. Ussing and K. Zerhan, 1951: "Active Transport of Sodium as the Source of Electrical Current in the Short-circuited Isolated Frog Skin," *Acta Physiologica Scandinavica* 23: 110-127). Because we have used the instrument extensively for frog skin experiments we have named it the frogometer. Among other desirable features of the design are (i) a self-contained power supply; (ii) meter overload protection; (iii) electrode imbalance compensation; and (iv) relative insensitiveness of operating parameters to variations in temperature and power-supply voltage.

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Components

The instrument is based on a commercially available operational amplifier (Model 3234/15, Burr Brown Research Corp.). This amplifier has field effect transistors at its input, with an input impedance reported by the manufacturer to be 10^{11} ohms—a value significantly higher than is available with measuring equipment (for example, inexpensive oscilloscopes) in typical teaching laboratories. For a biological membrane voltage of 0.1 volt the current flowing through the measuring circuit is about one picoampere. This current drain is so small that a frog skin potential, for example, can be measured continuously for hours without deleterious effect due to the frogometer. The operational amplifier can be mounted in a socket and thus easily removed and installed in another instrument whose controls are more suitable for other experiments (for example, a pressure transducer or thermistor for different physiological measurements). Of particular value is the relative indifference of this amplifier to power-supply voltage and to variations in that voltage. The power to operate the amplifier may be supplied by batteries of from five to 20 volts. Two batteries are required; they need not be of the same voltage. With batteries mounted inside the same metal case as the rest of the instrument, connection snarls are minimized, portable instruments can be designed, and power-supply noise is minimized. Battery life is prolonged by low current-drain (of the order of 1.2 mA) when the frogometer is operated as a voltmeter.

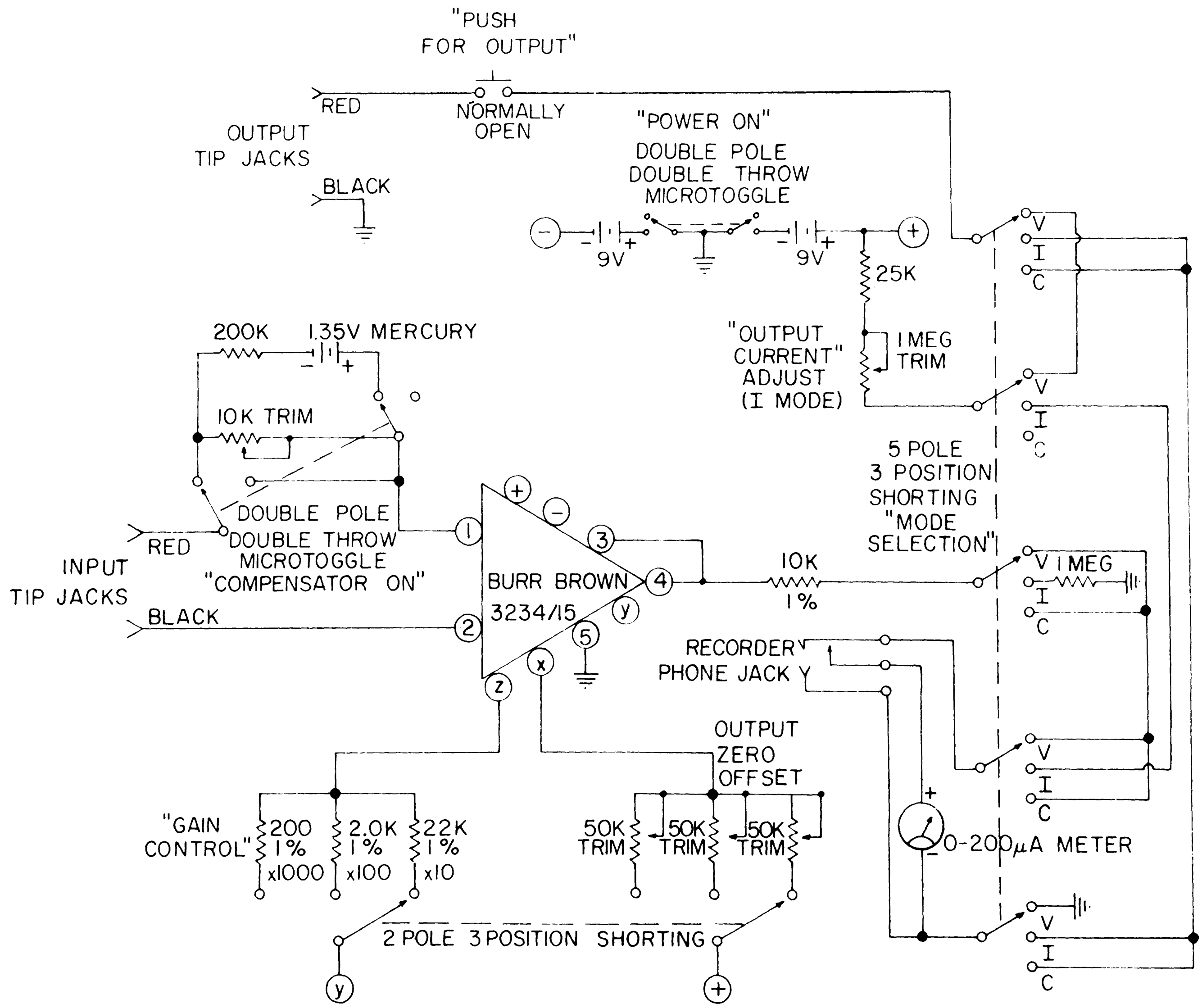

Fig. 1. Circuit diagram for the frogometer. The operational amplifier is mounted in a socket within the chassis.

The operational amplifier used has good common-mode rejection characteristics; that is, only differences of voltage between the input terminals are amplified, not variations in voltage that affect both inputs equally. Some biological voltage sources vary extremely rapidly. The amplifier used has good frequency-response characteristics and should be able to reproduce faithfully transients with time constants of the order of 10^{-5} seconds. Although this property is not of primary importance in measuring steady-state voltages across artificial membranes or living membrane organs, such as the frog skin, the instrument, with suitable display devices, could also be used to portray accurately most phenomena of neurophysiology.

Circuitry

The circuitry of the frogometer is shown in fig. 1. There are three operating modes available in the complete instrument: voltmeter *(V)*, microammeter current measure *(I)*, and voltage-clamping and current-measuring mode *(C)*. These are selected by a five-pole, three-position switch located on the panel.

In the *V* mode the biological source to be measured is applied across the input terminals of the amplifier, through an electrode imbalance compensator, and the output of the amplifier is connected through a 10,000 Ω resistor to a 0-200 µA meter. Amplifier gains of ×10, ×100, and ×1,000 are included in the present version of the frogometer, selected by a two-pole, three-position switch. The meter will read millivolts directly in this operating mode with full scale values of 200 mV, 20 mV, or 2 mV, depending on the amplification factor selected. (In classroom use some confusion had been experienced by students in interpreting down-scale readings, because the exact value of the voltage is not available. Substitution of a 100–0–100 microammeter may reduce these problems

Frogometer Parts List

1	Instrumentation amplifier model 3234/15 (Burr Brown Research Corp., International Airport Industrial Park, Tucson, Ariz. 85706.)
1	Amplifier socket (Burr Brown)
1	Chassis sloping 4 by 4 by 6 inches (BUD #AC-1612)
1	O-200 μA or 100-0-100 μA panel meter, 3½-inch face
2	red tip jacks
2	black tip jacks
1@	1.0 meg, 200 K, and 25 K (5%) dropping resistors (¼ watt)
1@	22 K, 2 K, and 200 (1%) precision resistors (¼ watt) for gain control
1	10 K (1%) precision resistor (¼ watt) for voltmeter circuit
3@	50 K multiturn trim potentiometers (¼ watt) (panel mount, screwdriver adjust) for output zero offset
1	10 K multiturn trim potentiometer (¼ watt) (panel mount with small knob) for electrode compensator adjust
1	1 meg multiturn trim potentiometer (¼ watt) (panel mount with knob) output current adjust (*I* mode)
1	5-pole, 3-position rotary switch (shorting) with knob for mode selection (Centralab PA-1018)
1	2-pole, 3-position rotary switch (shorting) with knob for gain selection and output zero offset (Centralab PA-1002)
2	Double-pole, double-throw micro toggle switch: power on, compensator on (Cutler Hammer)
1	pushbotton normally open for output
1	phono jack (¼-inch)
1	mercury cell and holder, 1.35 V (Mallory RM4R)
2@	9-volt transistor batteries, holders, and terminals

by making it unnecessary always to reverse the input connections to assess the magnitude of the down-scale voltage.)

Because the meter is driven by an amplifier of saturable output, the meter is protected against extreme overloads, which might burn it out. The maximum output voltage of the amplifier is the power-supply voltage minus four volts. Thus, for the nine-volt transistor batteries used in the frogometer, the maximum voltage that can be applied to the metering circuit is five volts—which, through the 10 K Ω resistor, would drive a maximum current of 500 μA: only a slight overload. This saturable output response must be kept in mind when using the frogometer to drive a recorder, since a wrong choice of recorder sensitivity may cause the saturation point to lie within the recorder's output trace and thus distort the recording.

Electrodes

Various electrodes may be used to detect the biological voltage and transmit it to the frogometer input. Either calomel electrodes (which are quite stable but expensive) or Ag–AgCl electrodes connected through agar bridges have been used. Our experience indicates that the use of calomel input electrodes may be worth their greater expense, inasmuch as they are more convenient to operate than are Ag–AgCl electrodes connected through agar bridges.

Because of the difficulty of obtaining perfectly matched electrodes, a compensator circuit has been included. This is essentially a voltage-dividing circuit driven by a small 1.35-V mercury cell. With the resistances used it can compensate electrode imbalances up to around 60 mV. In the configuration shown it is difficult to adjust smoothly the compensator for imbalances less than 2 or 3 mV. This is because of roughness of operation of the 10 K Ω trim potentiometer at very low resistances. In this situation, which is common with Ag–AgCl electrodes connected to a solution through agar bridges, it is best not to compensate the electrode imbalance; instead one should subtract it from the observed voltage. The compensator is arranged to drive the meter in the down-scale direction. In use the electrodes (or agar bridges) are immersed in the same solution, together with a grounding electrode (see below), and connected to the frogometer so that any voltage imbalance gives an up-scale meter deflection. By turning the compensator switch on and adjusting the 10 K Ω trim potentiometer, the voltage indicated may be reduced to zero. Compensation may have to be adjusted if the electrodes or agar bridges change their electrical properties over a period of time or if they are immersed in a solution of a composition different from that in which they were previously balanced. A sure sign that recompensation is necessary is a down-scale meter reading that persists when the electrodes' input connections are reversed. This is invariably due to excessive compensator voltage.

Amplification

The resistances connected between amplifier terminals "*y*" and "*z*" will allow amplification of ×10 (22 K Ω), ×100 (2.0 K Ω), or ×1,000 (200 Ω). The actual amplifier gain, according to the manufacturer, may be determined thus: gain = 1 + (200 K Ω/R). The maximum gain available is ×1,000. The actual in-

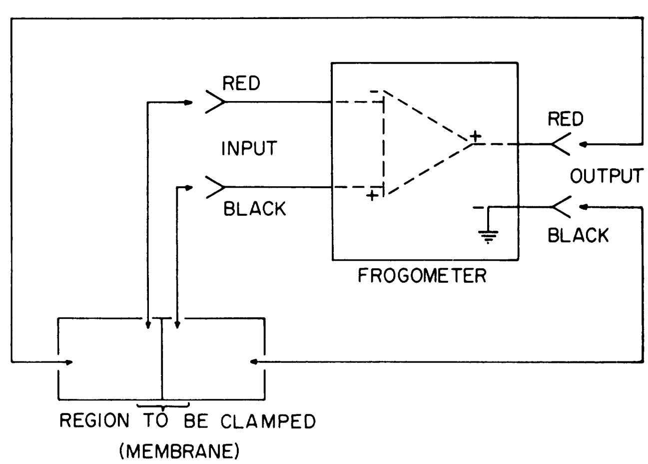

Fig. 2. Circuit diagram and experimental arrangement of the frogometer, input (calomel) electrodes, and output (Ag–AgCl) electrodes when operated as a voltage clamp. When operated as a voltmeter only, the red output electrode may be omitted.

dicated gains of a set of 16 such frogometers, constructed as shown in fig. 1, varied from the nominal values by no more than 10%. Using a calibrated voltage source it was possible to measure the true gain and calculate a correction factor that, when multiplied by the indicated voltage, would give the true voltage. If greater accuracy in gain setting is required, the indicated fixed resistors can be replaced by a smaller fixed resistor in series with a trim potentiometer of low resistance. The overall indicated gain then can be made as precise as the reproducibility of the indicating meter (usually ± 2%). The amplifier output is in general different from zero for a shorted input. The trim potentiometers connected between amplifier terminals "×" and "+" are included to adjust (from the panel) the output offset to zero for each gain setting. This setting tends to drift slightly as the batteries age.

Some users of the frogometer felt dissatisfied with the limited choice of gains, the steps being as large as a factor of 10. Additional intermediate gains can easily be included if the gain selector switch were to use more positions and additional resistors were installed. It should be noted, however, that most inexpensive meters are available with only one scale printed on the face.

A peculiarity of the operational amplifier circuit makes it necessary to have the noninverting input terminal (#2) connected to ground through a resistance of less than $10^4\ \Omega$ to allow the passage of small error currents. Failure to make this connection results in amplifier instability, which is manifested by a wildly swinging meter needle. This connection could have been made internally; but during operation as a voltage clamp it seemed desirable to separate the input from ground, except through the bathing solutions, to avoid passing any but the very minute measuring and error currents through the input electrodes. As a result of this requirement three frogometer terminals must be connected to the biological source when making a voltage measurement: the two input electrodes and the grounded (black) output electrode. In our use, Ag–AgCl electrodes have proved entirely satisfactory for output electrodes.

Use as Voltage Clamp

When the frogometer is being used as a voltage clamp it is used in the *I* and *C* modes. Manual clamping of a region between the output electrodes can be achieved by pressing the output pushbutton and adjusting the output current control until the meter (*V* mode) reads the desired clamped voltage. (See fig. 2 for experimental set-up). The current necessary to clamp the membrane (frog skin) to the desired voltage can be assessed by switching to *I*

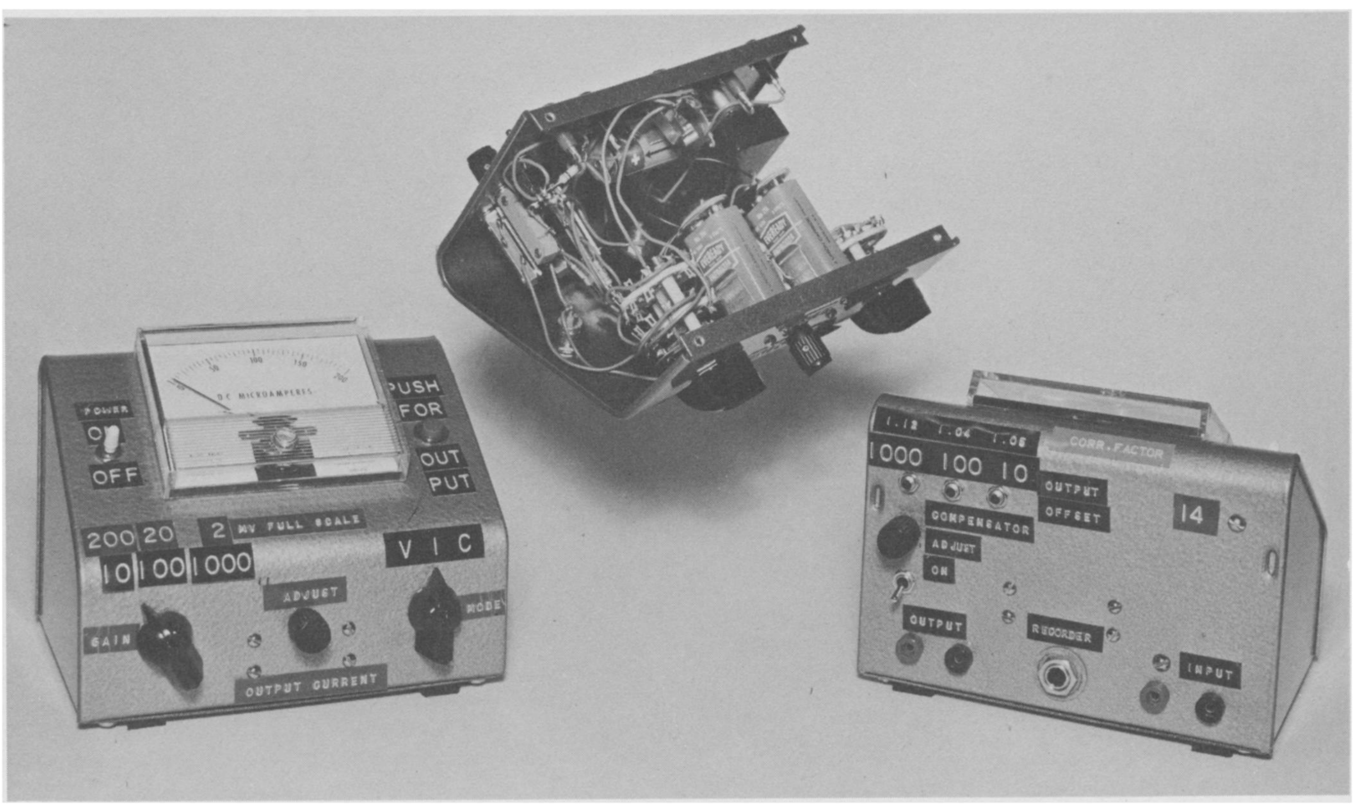

Fig. 3. Views of the front and rear panels of the frogometer and an interior view from the bottom, showing the arrangement of components on the chassis. The instrumentation amplifier is in its socket just forward of the two input tip jacks (right side of interior view).

mode and reading current directly from the meter in microamperes. No output current flows unless the pushbutton is depressed. This prevents the accidental exhaustion of the biological voltage source.

Although it is instructive for students to watch the change of membrane voltage during varying current-flow, experiments that require the continual assessment of short-circuit current become tedious. The *C* mode will cause the frogometer automatically to clamp a region to a predetermined voltage. Clamping occurs when the depression of the pushbutton allows current to flow from the amplifier output and through the microammeter to the output terminals of the frogometer. With a gain of 1,000 the typical frog skin may be clamped to zero to within a few 10ths of a millivolt. The presence of the input electrode compensator circuit allows clamping to voltages other than zero. As a simple example, assume the electrodes are imbalanced by 20 mV, red (#1) electrode negative. By adjusting the indicated 20 mV to 10 mV with the compensator, one requires the clamping current to drive the membrane to 10 mV (red side positive) instead of zero. With use of the compensator the clamping voltage can be adjusted from $(E_{IB} + 60)$ to $-(60 - E_{IB})$ mV, where E_{IB} is the electrode imbalance voltage in millivolts.

Following time-dependent voltage changes or short-circuit current changes is easier with a recorder. A phone-jack output has been included in the frogometer; this essentially substitutes the external recorder for the panel meter. Inexpensive recorders with low input impedances can be used; the frogometer protects the biological voltage source from overloading.

Capabilities and Costs

The frogometer has been used in the required cell-biology course for sophomores at the University of Oregon for two years. The students seem responsive to and challenged by the experiments. We have been using the instrument to perform experiments somewhat similar to those described by L. Packer in *Experiments in Cell Physiology* (1967: Academic Press, New York) p. 191 ff. The instruments constructed in the science services electronics shop of the University of Oregon cost about $250 each for parts and labor. The parts, including the amplifier, cost about $180. This is much less expensive than a potentiometric recorder that would not have voltage clamping ability.

Acknowledgments.—Don Kitselman and the electronics shop of Science Services, University of Oregon, made the layout of components and constructed and tested the 16 frogometers on which the article is based. The circuit diagrams were drawn by Shiela Finch. The photographs were taken by Harrison Howard and Carol Cogswell. The cost of the frogometers was supported by grant GY-5153 from the National Science Foundation.

N.S.F. SUMMER INSTITUTES

High school teachers will study such subjects as ecology, environmental assessment, the population explosion, and the nation's need for power and its impact on the environment at 1972 summer institutes supported by the National Science Foundation.

To assist schools to improve their effectiveness of instruction in science and mathematics, NSF has awarded grants totaling $13.7 million for summer institutes for secondary-school teachers of science and mathematics. The grants support 274 institutes that provide study opportunities for 10,000 high school teachers throughout the country.

The institutes, to be conducted by 190 colleges and universities, usually last from six to eight weeks. A typical institute enrolls about 40 participants and includes laboratory or field work, lectures, discussion sessions, and seminars.

For each participating teacher NSF provides a stipend of up to $75 a week, with supplementary allowances for dependents and travel. Participants pay no tuition or fees.

A *Directory* listing institutions offering summer institutes for secondary-school teachers and supervisors may be obtained by a postcard request to Summer Study Program, Division of Pre-College Education in Science, National Science Foundation, Washington, D.C. 20550. Applications must be submitted to the various institutes by March 1, 1972.

ONE MAN'S PROTEST

A steelworker in the Jones & Laughlin Steel Corp.'s Cleveland plant was suspended when he refused to dump oil, solvents, and other wastes into the Cuyahoga River, according to United Press International. But Gilbert Pugliese got the backing of his United Steelworkers of America (AFL-CIO) local, which threatened a wildcat strike at the plant unless the company reinstated the employee immediately. Jones & Laughlin rescinded the five-day suspension and paid Pugliese for lost time. "I'm surprised and elated," said Pugliese, "over the kind of support I got from my fellow workers, the union, and the public."

Beautify Your Waterfront

Dozens of beautification ideas are presented in a 68-page publication, *Waterfront Renewal,* dealing with urban waterfronts and what has been done with them in Wisconsin. Write to the Bureau of State Planning, Wisconsin Department of Administration, 1 W. Wilson St., Madison.