Kurzmitteilungen und Methodisches

Voltage Clamp of a Small Muscle Membrane Area by Means of a Circular Sucrose Gap Arrangement* **

M. HENČEK, W. NONNER, and R. STÄMPFLI

Institute of Normal and Pathological Physiology SAV, Bratislava, and I. Physiological Institute of the Medical Faculty, Homburg (Saar)

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Summary. A new method is described which permits the measurement of membrane currents of thick muscle fibres (diameter 300 μm or more) of Astacus fluviatilis or Balanus balanus under voltage clamp conditions.

The potential difference across a small patch of membrane (60—100 μm in diameter) is controlled by connecting a voltage source across it with two external electrodes. One of them is connected to the fluid bathing the muscle fibre. The other, tubular one is in touch with the test area. The current flowing through the electrodes represents the sum of the membrane current flowing across the test area and the leak current flowing in the external fluid between the electrodes. In the first version of the method the leak current is limited by a circular sucrose gap around the test area. In the second, more elaborate method, the leak current is eliminated by a system of two concentric sucrose rings with a guard ring electrode between them. This method permits in addition the measurement of full sized action potentials in the test area.

Key-Words: Voltage-Clamp — Giant Muscle Fibre — Membrane — Current Clamp — Sucrose Gap.

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The sucrose gap method (Stämpfli, 1954) has been widely used to limit the membrane area used in current or voltage clamp experiments. Julian, Moore and Goldmann (1962) were the first to make "artificial nodes" by reducing the electrically accessible part of lobster axon to a narrow ring by applying two sucrose gaps.

If such arrangements are used with muscle fibres, one of the great inconveniences is the contracture which occurs as soon as the surface of the muscle membrane is washed with isotonic sucrose solution. This difficulty is particularly marked in experiments with giant muscle fibres of Crustacea or Cirripediae and, furthermore, contact with sucrose solution produces a rapid deterioration of these fibres. Therefore, it seemed desirable to develop a variation of the method, so that a minute patch of membrane could be isolated by a circular sucrose gap of very small width.

The equivalent circuit used in this arrangement is represented in Fig. 1. The inside of the membrane with its capacity and resistance \((C_m \text{ and } R_m)\) is connected through a series resistance with a capacity

![Fig. 1. Schematic drawing of the principle of the new method: \( S \) Source of clamp potential; \( R_0, C_0 \) resistance and capacity of the "internal" part of the circuit including the membrane capacity \((C_o)\) and the membrane resistance in series with the resistance of the myoplasm \((R_0)\) with the exception of the small membrane patch, surrounded by sucrose \((C_m \text{ and } R_m)\). The external part of the circuit consists of the external electrode and the current measuring device. The electrode resistances are not drawn, being much smaller than the 100 kΩ resistor used for measuring the voltage drop produced by current flow. \( V \) and \( I \) beams of Tektronix oscilloscope used for recording the potential and current. \( R_s \) parallel (leak)-resistance across annular sucrose gap. An intracellular micropipette is drawn to show how the stability of an applied step potential and the influence of the series resistance can be checked.](image-url)