Chapter 2

Voltage clamp techniques

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1. Introduction

Much of what we know about the properties of ion channels in cell membranes has come from experiments using voltage clamp. In general, the method allows ion flow across a cell membrane to be measured as electric current, whilst the membrane voltage is held under experimental control with a feedback amplifier. The method was first developed by Cole (1949) and Hodgkin *et al*. (1952) for use with the squid giant axon. Since then, many variants of the technique have evolved and voltage clamp analysis has been extended to a wide range of tissues.

The usefulness of the voltage clamp stems firstly from the fact that it allows the separation of membrane ionic and capacitative currents. Secondly, it is much easier to obtain information about channel behaviour using currents measured from an area of membrane with a uniform, controlled voltage, than when the voltage is changing freely with time and between different regions of membrane. This is especially so as the opening and closing (gating) of most ion channels is affected by the membrane potential.

This chapter will concentrate on voltage clamp methods that are used to measure currents from whole cells or large areas of membrane containing at least a few hundred channels; such currents are usually called macroscopic currents. We shall give particular attention to the single and double microelectrode methods. The use of both these techniques is increasing; double microelectrode clamp is being used for studies of channels expressed in *Xenopus* oocytes, while the switched clamp circuitry used for single microelectrode clamp is sometimes used with whole cell patch pipettes because it provides a neat way of dealing with the series resistance problems that can arise with such electrodes. The patch clamp technique has extended the application of voltage clamp methods to the recording of ionic currents flowing through single channels, but in its whole cell configuration has also become the most

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widely used method for recording macroscopic currents. Whole cell patch clamp is mostly described in Chapter 5, though we shall mention the application of some of the general principles of voltage clamp to the technique in the present chapter.

2. Theory

The basis of the voltage clamp may be understood by consideration of the simplified equivalent circuit for the cell membrane shown in Fig. 1A. Here, C is the membrane capacity while the channels that allow ionic current, I_i, to flow through the membrane are represented by the variable resistor R. The current I_m flowing through the circuit will be the sum of I_i and a capacity current,

$$I_m = I_i + C\frac{dV}{dt}$$

In voltage clamp experiments the voltage is usually forced to change in a square step fashion, being changed as rapidly as possible from one steady level to another (Fig. 1B). Under these conditions a brief spike of capacity current flows at the edges of the pulse, but when the voltage is steady dV/dt is zero and so the capacity current is zero. The ionic current may therefore be obtained free from capacity current once the change in voltage is over. In most experiments it is ionic current which is measured to give information about the permeability properties of channels and the mechanisms by which they open and close, though some studies concentrate on components of the capacity current which are related to channel gating.

For the voltage clamp of a particular preparation to be adequate the system used should meet certain criteria for the accuracy of voltage control, speed, and isopotentiality.

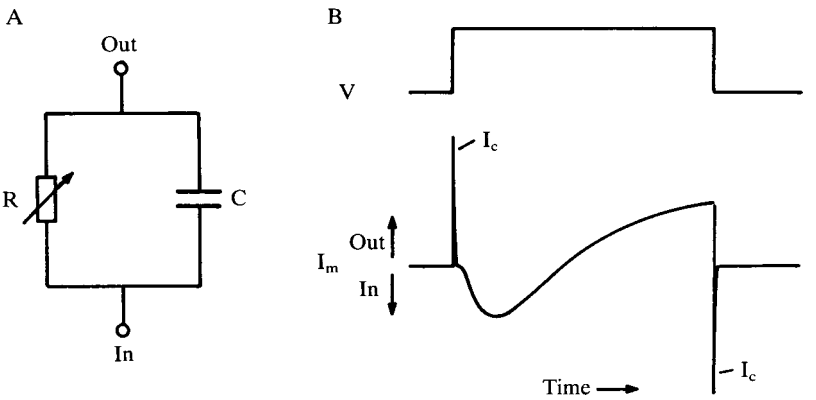

Fig. 1. (A) Simple electrical analogue of the cell membrane. (B) Typical currents recorded from a voltage clamped excitable cell when the membrane potential is stepped in a square fashion to a level at which ionic channels open. Spikes of capacity current occur at the edges of the pulse; during the pulse inward, and then outward ionic currents flow. The time course is typically in the order of a millisecond.

Control of membrane potential

The accuracy with which the membrane voltage is controlled depends on having sufficiently high gain in the clamping amplifier. This can be seen by considering the schematic voltage clamp circuit of Fig. 2, as discussed by Moore (1971). The membrane potential, V_m, is measured by the voltage follower, which has very high input impedance and so draws negligible input current. The clamping amplifier, of gain A, compares V_m with the command potential E, and passes current through the access resistance R_a (which might consist of an electrode and the cytoplasmic resistance) to control V_m. The output of the clamping amplifier, V_o, is given by

$$V_o = eA = A\,(E - V_m)$$

This output is divided between the access resistance and the membrane (for the moment we will assume that R_s, the series resistance, is zero), so for a current I

$$V_o = V_m + R_a I$$

Substituting for V_o and rearranging gives

$$V_m = E\,\frac{A}{1+A} - \frac{R_a I}{I+A}$$

Thus, as the gain A is increased, the membrane potential approaches the command potential more closely, and the effect of the access resistance is reduced.

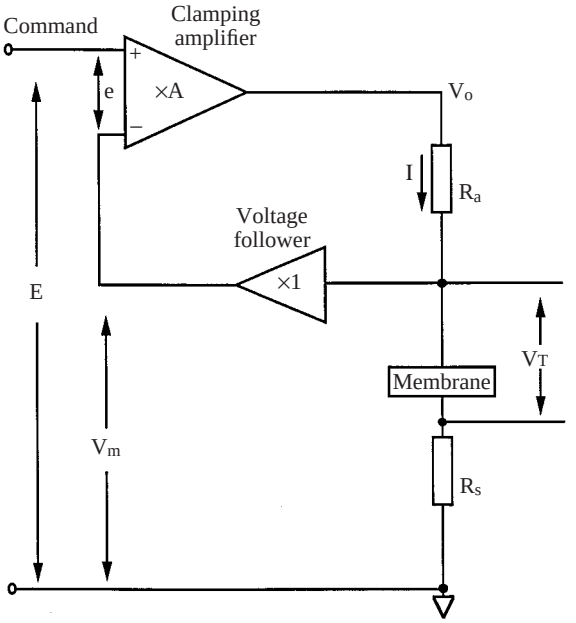

Fig. 2. Simplified schematic voltage clamp circuit (after Moore, 1971).

Series resistance

In practice, there is normally also a resistance in series with the membrane and between the voltage recording electrodes. This is represented by R_s in Fig. 2. When a current I flows across the membrane this resistance leads to a discrepancy between the measured membrane potential V_m (which is what the clamping amplifier controls) and the true potential difference across the membrane, VT. The size of the error is $I \times R_s$, so that the problem is most likely to be serious when large membrane currents are flowing. The usual approach is to measure R_s (see below) and so decide whether the error IR_s is significant. If so, compensation for R_s may be achieved by adding a voltage signal proportional to the membrane current, and scaled appropriately, to the command voltage of the clamping amplifier. Usually the level of compensation is set using a potentiometer and compensation for around 80-90% of the measured R_s is possible, with attempts to increase the level further driving the clamp circuit into oscillation. An example of R_s compensation in a two-electrode clamp circuit is given later in this chapter.

Series resistance is usually measured under current clamp conditions by applying a step change in current, I. Theoretically, the voltage response first shows a discontinuous jump of size IR_s, followed by a rise in voltage with initial slope equal to I/C, where C is the membrane capacitance. Under experimental conditions, with a current step of finite risetime, it is unfortunately often quite hard to distinguish the size of the initial voltage jump. This problem, and a method of correcting for the risetime of the current step, have been discussed by Binstock *et al.* (1975).

Series resistance can often be a problem in whole-cell patch clamp experiments. In this technique, described in Chapter 4, the patch pipette is used both to record voltage and as the path for current flow into the cell. This means that the access resistance of the pipette, usually in the order of a few MΩ, contributes to the series resistance. Permeabilized patch whole cell recording methods using nystatin or amphotericin B usually give access resistances 2- to 3-fold higher, with correspondingly greater possible problems due to R_s. For these reasons it is important to measure Rs in whole cell patch clamp experiments, as described in Chapter 4, and to calculate from the maximum size of the currents being studied whether the resulting voltage error will be serious. If so, a good proportion of R_s can usually be compensated using circuitry provided in most commercial patch clamp amplifiers. In extreme cases, it is also sometimes possible to check the true membrane potential of the cell with a separate microelectrode.

Clamp speed

The clamp should be able to change membrane potential sufficiently rapidly for the capacity current transient to be over by the time that ionic current is measured. Clearly, this criterion is most severe when fast ionic currents are of interest.

Voltage clamp systems have at least two, and usually more, time constants in their feedback circuit, and such systems tend to oscillate as gain is increased (see e.g. Moore, 1971). Generally, the experimental aim is for something close to a critically damped response, in other words the fastest rise in voltage which just avoids

oscillation, or a slightly underdamped response, which is faster but has a slight voltage overshoot. Lags in the system will be caused by the need to charge the membrane capacity through an access resistance, and by the time constant(s) of the control amplifier itself. There will also be a delay in the measurement of membrane potential caused by capacitance at the input of the voltage follower, and at the microelectrode if one is used. This delay may be reduced by use of a voltage follower with capacity compensation.

The gain of the control system needs to be high to ensure good voltage control. Katz & Schwarz (1974) have given a theoretical analysis of the conditions for a critically damped response with a simplified voltage clamp circuit (see also Smith *et al.* 1980). They point out that with high gain, critical damping is achieved either when τ_L, the time constant of the membrane-electrode-solution load, is considerably greater than τ, the amplifier time constant, or when the converse is true. For systems with low resistance electrodes (for example axial wires) $\tau \gg \tau_L$ will generally apply, whereas $\tau_L \gg \tau$ will apply for microelectrode clamps. These authors also describe the use of current feedback to reduce the effect of access resistance. In summary, the frequency response of the control system needs to be adjusted for the particular preparation to be clamped, and gain and capacity compensation are adjusted to give the best risetime obtainable.

Isopotentiality (space clamp)

Membrane current should be recorded from an area of uniform potential, so that the current comes from a population of channels that are all experiencing the same voltage. How fully this criterion is met depends on the geometry of the preparation and electrode system used. With axial wire clamps an internal wire electrode ensures isopotentiality of a length of cylindrical axon or muscle fibre. Microelectrode clamps, which deliver current at a single point, will give a good clamp in round cell bodies, though attached structures such as axons and dendrites may not be controlled. Whole cell patch clamp is often applied to small cells where very good space clamp is achieved, though again long processes may not be controlled. In some cases a clamp which controls voltage at one point only may be sufficient to examine currents from the limited area of membrane that is clamped. An example is the two electrode point clamp of the motor endplate of skeletal muscle (Takeuchi & Takeuchi, 1960). Currents arising from electrical activity of unclamped or poorly controlled regions of membrane can often be recognized as ‘notches’ of inward current which may occur a relatively long time after the start of a depolarizing voltage step, and correspond to a very abrupt rise in the current-voltage relation for inward current.

3. Techniques and their applications

A variety of different voltage clamp methods are shown in Fig. 3. The choice of method depends largely on the size and shape of the preparation to be investigated and is discussed below. In general the methods all have a feedback amplifier which

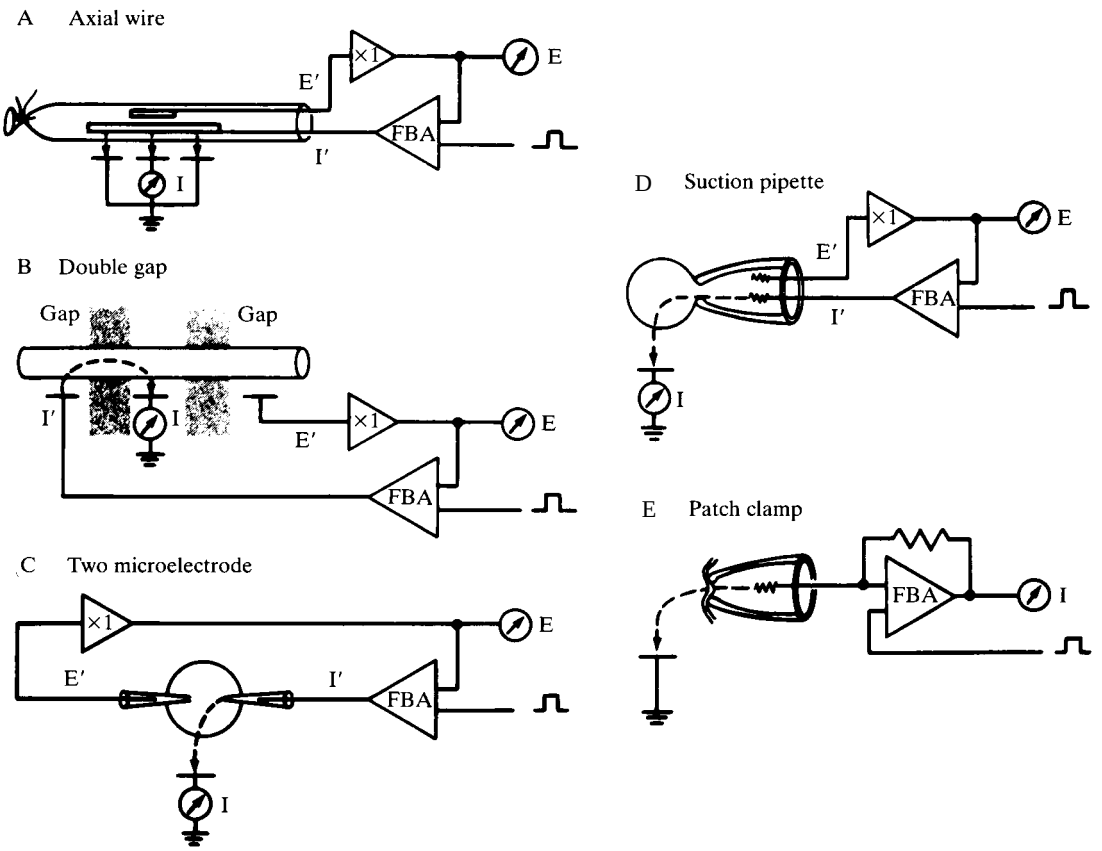

Fig. 3. Voltage clamp techniques. In each case FBA is the feedback (or clamping) amplifier, E′ is the voltage electrode and I′ is the current electrode. (Reproduced with permission from Hille, 1984).

receives a signal from a voltage recording electrode, E′ and compares this with a command potential. The difference between these signals is amplified and applied to the membrane as a current via a current electrode I′. In the circuits illustrated the current I is recorded at the electrode that grounds the bath; it may also be recorded as the voltage drop across a resistor in series with the current electrode, or across the resistance of the external solution. The patch clamp circuit shown operates rather differently, being essentially a current-to-voltage converter.

Axial wire methods

These methods are applicable to large cylindrical preparations such as giant axons of squid and *Myxicola* and barnacle giant muscle fibres. The internal voltage and current electrodes are formed by wires inserted longitudinally down the centre of the preparation, though sometimes a separate measurement of membrane potential is made with a microelectrode. The method gives a very rapid clamp as the access resistance is low, and is often combined with perfusion methods to control the

internal solution (see Baker, 1984). Descriptions may be found in Hodgkin *et al.* (1952), Cole & Moore (1960) and Chandler & Meves (1965).

Gap methods

These methods are also applicable to elongated preparations, though their diameter does not have to be large as it does for axial wires. Thus the method has been used, for example, for studies on myelinated axons (Nonner, 1969), and vertebrate muscle fibres (Hille & Campbell, 1976). The preparation runs through a number of pools of solution separated by air, sucrose or vaseline gaps of high electrical resistance. The end pools usually contain intracellular solution and the cut ends of the preparation lie in them. These pools allow measurement of membrane potential and delivery of current.

A rather different type of gap method can be used for large spherical cells. In these techniques the cell is held in a funnel shaped hole in a partition separating two solution-filled chambers, where it seats against the walls to form an electrical seal. The cell membrane is broken or permeabilized on one side so as to give access to the inside of the cell. Thus the cell is clamped between two pools of solution, one in contact with the cell exterior, and the other with the cytoplasmic solution. This method has been used with molluscan neurones (Kostyuk & Krishtal, 1977), and an elegant system of this type has recently been developed to give a rapid low noise clamp of *Xenopus* oocytes for the study of gating currents of channels expressed from mRNA (Tagliatela *et al*. 1992).

Microelectrode clamps

In these techniques, microelectrodes are used both to measure the membrane potential and to deliver the current that controls it, resulting in a rather high access resistance. Since current is delivered at a point, the method can be used to space clamp cells which are approximately spherical or are short cylinders. Alternatively, a point clamp may be obtained in a long cell; for example in the endplate region of a muscle fibre. A special case is the three electrode clamp (Adrian *et al.* 1970; Stanfield, 1970) which was developed for use at one end of a muscle fibre. Two electrode clamps can be applied to cells large and robust enough to tolerate two intracellular electrodes (e.g. Meech & Standen, 1975; Adams & Gage, 1979; Smith *et al.* 1980). Smaller cells may be too fragile to allow the insertion of two electrodes without causing damage. Alternatively, the cell under study may not have been impaled under direct visual control, so that the placement of a second electrode is impracticable. In these circumstances a single electrode voltage clamp may be employed. The first of two available recording strategies was originally employed by Wilson & Goldner (1975) and involves switching the microelectrode rapidly (at 3-20 kHz) between voltage recording and current passing modes with a clamp feedback amplifier controlling the amount of current delivered. Such methods have been applied to study kinetically slow membrane conductances in central neurones (Johnston *et al.* 1980; Halliwell & Adams, 1982) and to analyse faster synaptic currents in the central nervous system (Brown & Johnston, 1983; Finkel & Redman,

1984). Another application of the single electrode clamp has been to control voltage and measure macroscopic current in neurones whilst leaving a second microelectrode free to make intracellular injections (Adams *et al.* 1982). Despite these advantages the switching single electrode clamp is inferior to two electrode configurations in terms of its slow voltage response time and the introduction of noise into the recording of both current and voltage, which is inherent in the manner the clamp system is implemented: for fuller description of the technique and its optimization the reader is referred to Finkel & Redman (1984) and to the last section of this chapter. A second voltage-clamp strategy was elaborated by Blanton *et al.* (1989) for situations where neurones cannot be visualised directly when it became clear that it is possible to use the whole-cell variant of the patch clamp technique beneath the surface of a brain slice preparation. The use of lower resitance patch pipettes in conjunction with switching techniques has improved noise levels and reduced series resistance errors in a variety of experimental situations, one of which is illustrated below.

Suction pipette methods

In these methods a cell is sealed to a pipette that has a fairly large tip orifice (up to 50 μm). The membrane within the opening is destroyed, giving low resistance access to the interior of the cell. The pipette may be used both to pass current and to record potential, though the series resistance provided by the pipette can cause problems in the voltage measurement. Sometimes a microelectrode or a second suction pipette is used to overcome this difficulty. A clamp that switches between voltage recording and current passing, as used for the single microelectrode method, may also be used to overcome this series resistance problem, since with this system no current flows at the instant when voltage is sampled. Suction pipette methods have been used with preparations that can be dissociated to give isolated cells, for example neurones and cardiac myocytes, and are usually used to perfuse the interior of the cell as well as to provide voltage clamp. Accounts of suction pipette techniques may be found in Byerly & Hagiwara (1982) and Kostyuk & Krishtal (1984).

The whole-cell form of the patch clamp technique uses a pipette with a smaller tip (usually 1 μm) and can be applied to many types of cells which are too small for other voltage clamp methods.

4. Practice and experimental details

This section will be restricted to descriptions of the two microelectrode and single electrode voltage clamp methods.

Two electrode voltage clamp

Fig. 4 shows a schematic diagram for a two electrode voltage clamp circuit, and an example of a circuit for a clamping amplifier is described in the appendix to this chapter and illustrated in Fig. 7. The detailed methods described here refer to clamping molluscan giant neurones, though the methods for other preparations such

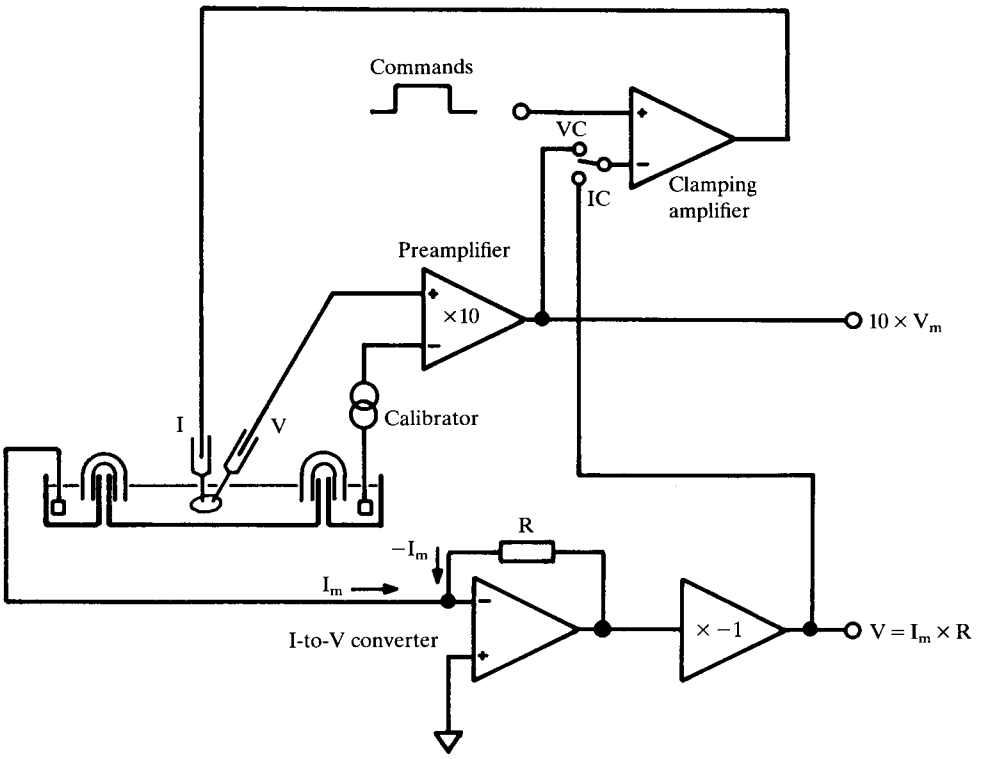

Fig. 4. Two electrode voltage clamp circuit. The circuit may be switched either to clamp voltage (VC) or current (IC).

as oocytes are essentially the same. Several commercial amplifiers are available for two electrode voltage clamp. These are made by, for example, Axon Instruments (Axoclamp or Geneclamp), NPI, or WPI (S7070A or OOC-1).

A schematic diagram of the clamp circuit is shown in Fig. 4. The cell is penetrated with two microelectrodes, one to record voltage and the other to pass current. A preamplifier records membrane potential and the clamping amplifier passes current to control this potential.

Electrodes and voltage recording. The microelectrode resistance is made as low as possible while avoiding excessive damage to the cell. Thin-walled glass may be used to obtain a low resistance for a given tip size; for molluscan neurones voltage electrodes of 5-10 MΩ and current electrodes of 1-2 MΩ are commonly used.

The voltage electrode is mounted in a holder containing an Ag/AgCl pellet, and plugs directly into one headstage of the preamplifier. A second headstage follows the bath potential via an Ag/AgCl pellet and agar bridge, and the preamplifier records differentially between these two inputs with a gain of 10. The electrode is shielded by wrapping in aluminium foil to within 1-2 mm of the tip, and then insulating this foil from the bath with parafilm and vaseline. The shield both reduces capacity coupling between current and voltage electrodes, and, since it is connected to the driven

screen, which provides capacity compensation for the preamplifier, also increases the speed of voltage recording. Other shielding methods include coating the electrode with conductive paint, and some workers also use a grounded shield around the current electrode or between the two electrodes. Keeping the level of the bath solution as low as possible also reduces coupling between the electrodes.

The voltage signal from the preamplifier is fed to the clamping amplifier, and also to an oscilloscope, pen recorder, and bleater (voltage-to-frequency converter plus audio amplifier).

Clamping amplifier. This is a differential amplifier with a gain up to 10 000 which may be varied at two different stages. The circuit used (see Appendix) also has a high voltage (~120 V) amplifier in its output stage. Its frequency response may be varied by altering the capacity in the feedback stage of the differential amplifier.

Command pulses and holding potential. Command pulses are derived from a digital pulse generator and are fed into the clamping amplifier input via a summing amplifier, which also receives an offset voltage for zeroing the amplifier. Because of the ×10 gain of the preamplifier, the command voltage must be 10 times the desired voltage step. The command summing amplifier is followed by a variable low-pass filter, which may be used to reduce the risetime of the command voltage step. This often makes it easier to obtain a fast voltage clamp step while avoiding oscillation.

To set the holding potential a steady voltage signal can be applied to the command summing amplifier in the same way as command pulses. An alternative is to use a precision voltage source (the calibrator of Fig. 4) in series with the bath electrode and so provide an accurate holding potential. Commercial clamping amplifiers usually provide an inbuilt potentiometer to set the holding potential.

Measurement of membrane current. Membrane current is measured via a bath electrode which is held at virtual ground by a current-to-voltage converter circuit (Fig. 4). The operational amplifier strives to keep its two inputs at the same potential, in other words to keep its negative input at ground. To do this it must pass a current equal to $-I_m$ around its feedback loop, so that the voltage at its output equals $-I_m R$. Thus with a feedback resistor of 1 MΩ the circuit will give 1 mV.nA^{-1}. The operational amplifier should have a high input impedance (>10^{12} Ω) so that no current flows into its negative input. For this reason an amplifier with an FET input is used. The whole control circuit may be made to clamp current, rather than voltage, by feeding the output of this current-to-voltage converter to the clamping amplifier.

Series resistance compensation. The voltage output from the current-to-voltage converter may also be fed to a potentiometer, which adds a proportion of the signal back to the clamping amplifier command input to compensate for R_s. For example, a series resistance of 10 KΩ will give a voltage error of 10^{-2} mV per nA of current, so that this voltage should be added to the command voltage. In the present circuit, this would be achieved by adding 1/10 of the output of the I-to-V converter (1 mV.nA^{-1}) to the command voltage; this allows for the effective division of command voltages by 10.

Leakage and capacity compensation. It is possible to provide analogue compensation for linear leakage current through the membrane, and for most of the

capacity current. For leakage compensation, a suitable proportion of the membrane voltage is subtracted from the current signal before it is fed to the oscilloscope or other recording apparatus. For capacity compensation, the voltage is differentiated, and the resulting signal is subtracted. Often two or three differentiators with different time constants are used.

Experimental procedure. With the control circuit switched to current clamp, or with the clamping amplifier disconnected from the current electrode, a cell is first penetrated with the voltage electrode. The calibrator is used to back off the resting potential, and so provide a holding potential for the voltage clamp. The current electrode is then inserted; this seems to be aided by a current pulse repeated at about 1 Hz. The gain of the clamping amplifier is set low and the voltage clamp switched on. The rise time of the clamp is then increased by increasing the gain and adjusting the capacity compensation of the preamplifier, while observing the response to a small hyperpolarizing command pulse.

Single electrode voltage clamp

In Chapter 1, an alternative to the 'bridge balance' technique is presented for the elimination of the voltage drop across the resistance of an impaling microelectrode, whilst the potential of a cell is being changed by the passage of current through the electrode. In essence, this scheme is the basis of the method proposed by Brennecke & Lindemann (1974a,b) and implemented independently by Wilson & Goldner (1975). Fig. 5A is a schematic diagram of the Wilson and Goldner system, which is the technique elaborated in commercially available amplifiers and most home-built devices. A_1 is a high input-impedance high speed amplifier to which the impaling microelectrode is connected *via* a non-polarizable Ag/AgCl half-cell. A_1 measures the voltage, with respect to ground, of the electrode shank: this potential comprises the membrane potential (V_m) of the impaled cell *plus* the voltage drop (V_e) across the resistance of the electrode caused by current (I_o) injected into the cell through the microelectrode. This current is supplied from a controlled current source or pump (CCS) to the input of A_1 *via* a 2 MΩ resistor. The input resistance of A_1 being very large ($>10^{11}$ Ω), the current from the CCS passes down the electrode into the cell. The output of A_1 is fed to a sample and hold device (SH_1) which in turn is connected to A_2, the clamp feedback amplifier, where, after sampling in SH_1, the voltage is compared with the clamp command potential (V_c). When operating in voltage-clamp mode, the output of A_2 is led *via* the electronic switches S_1 in a feedback loop to control the output of the CCS. The gain of the system is controlled by adjusting the sensitivity of the CCS to its input voltage. It will be noticed that the voltage at the input of A_1 is the sum of V_e+V_m; in order that the cell be clamped at its true potential, V_e has to be eliminated. This is accomplished by electronic control of the state of switch S_1 in order to pass current discontinuously. Consider the feedback loop closed by S_1 being connected to A_2: the output of A_1 = V_m+ V_e. If S_1 changes state, I_o becomes zero and V_e decays to zero with the time constant = $R_e.C_s$, where R_e is the microelectrode resistance and C_s is the associated stray capacitance (see Chapter 1).

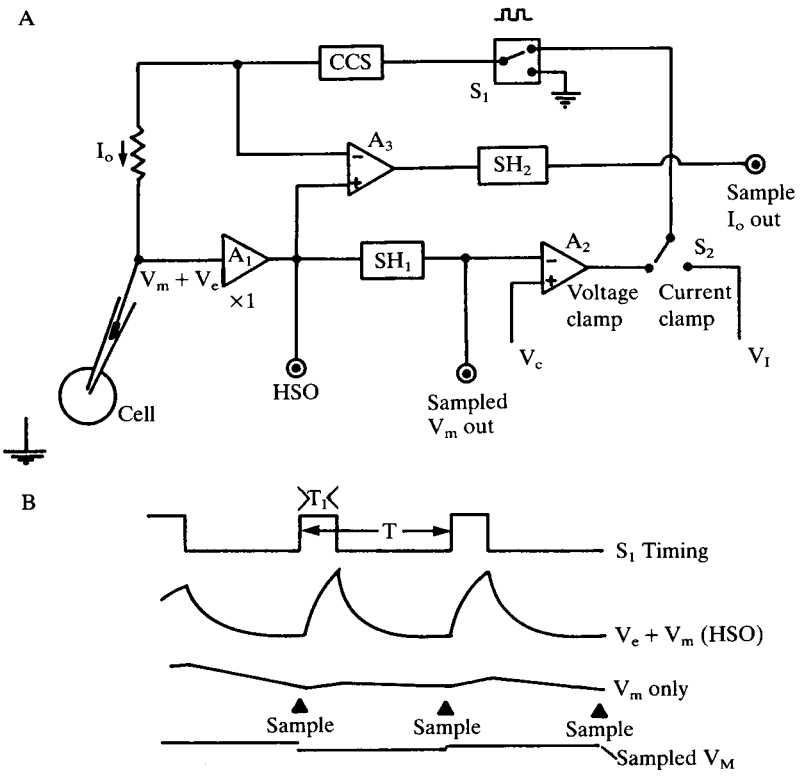

Fig. 5. (A) Schematic diagram of single electrode clamp circuit. (B) Diagram showing the timing of the states of switch S_1 and the corresponding signals recorded at HSO and SH_1 (sampled V_m). Also shown is the varying theoretical potential on the cell membrane alone (V_m only) and the instants at which SH_1 samples the potential at HSO. T is the cycle time and T_1 is the time for which current is passed. Note that duty cycle = $T_1/T \times 100$ %. See text for further details.

If SH_1 samples when the decay of V_e is complete, the output of SH_1 equals the true cell membrane potential, which will have been changed slightly, owing to charge transfer onto the membrane capacitance during current injection. When S_1 changes again, another current injection is made, based on the difference between the new value of V_m and V_c. The timing of the states of S_1 (see Fig. 5B) is such that current is regularly injected for between 25-50% of the total time (25-50% duty cycle) and the sample point of SH_1 is such that V_e has decayed completely before an upgraded current injection is re-imposed. (In practice, the sample point of SH_1 is timed to allow maximum decay of V_e: i.e. it occurs *just* before the current injection period). Thus the cell is clamped close to the value of V_c by reiterating this procedure at high frequency (3-20 kHz).

The main limiting factor determining the maximum frequency at which the system will run is the decay time of the microelectrode voltage signal. To reduce this the amplifiers are furnished with efficient capacity compensation circuitry, but attention must be paid to minimizing both the electrode resistance and the capacitance to

ground that cannot be compensated completely (for example, capacitance distributed within the immersed shank of the microelectrode (see Chapter 1)). Other considerations required in operating this clamp system are: (i) that the cell membrane time constant should be long (>10^2 times greater) compared with the time constant of the recording electrode: this ensures that the charge transfer during current injection is effective in changing V_m; (ii) that the switching frequency is at least twice the frequency component of the fastest current that is to be resolved (in accord with Sampling Theory).

Recording current and voltage. Reference to Fig. 5 will indicate that the output of SH_1 should approximate true V_m: the faster the switching of cycling rate, the better the approximation will be. This value is read out to a voltage recording device. Current injected into the cell is measured by observing the voltage drop across the 2 MΩ resistor with amplifier A_3. Because of the discontinuous current delivery, this voltage is also sampled with a sample and hold device SH_2. The sample timing is not critical; any time during the current injection period will suffice. However, because current is passed for only part of the cycle, the signal from SH_2 is scaled by a percentage factor equal to the duty cycle (e.g. for 50% duty cycle it is halved, for 25%, divided by four and so on). This arises because the membrane capacitance stores the electrical charge transferred and the current is charge transferred per unit time. The scaled signal from the current monitoring amplifier is also read out to a recording device to measure current.

Use of single electrode clamp in other modes

(i) Discontinuous current clamp. By changing the state of switch S_2 (Fig. 5A) the feedback loop of the voltage clamp circuit may be broken and the CCS be brought under control of an external current injection command potential (V_I). Provided, as before, that attention is paid to the frequency of switching such that adequate time is allowed for the decay of the potential V_e then V_m will be registered faithfully at the SH_1 output. In this configuration the cell will be under conditions of current clamp.

(ii) Conventional bridge-balance operation. Incorporated in most instruments using the above techniques is the facility to disconnect the circuitry for switched current injection, together with the sample and hold devices, and pass continuous current from the CCS. In this case, an appropriately scaled proportion of the command potential V_I (equal to the voltage drop V_e) is subtracted from the output of A_1 to eliminate V_e. This has been discussed in more detail in Chapter 1.

Experimental application of single microelectrode clamp with sharp and other microelectrodes

The input amplifier and the final stage and output resistor of the CCS are contained in a small probe that is mounted close to the preparation and into which is connected the electrode holder. The electrodes used demand the same characteristics and treatment for reducing stray capacitance as mentioned above for 2 electrode clamping (i.e. the lowest resistance electrode compatible with experimental aims and the minimum non-compensatable capacitance). Because sharp electrodes employed with the single

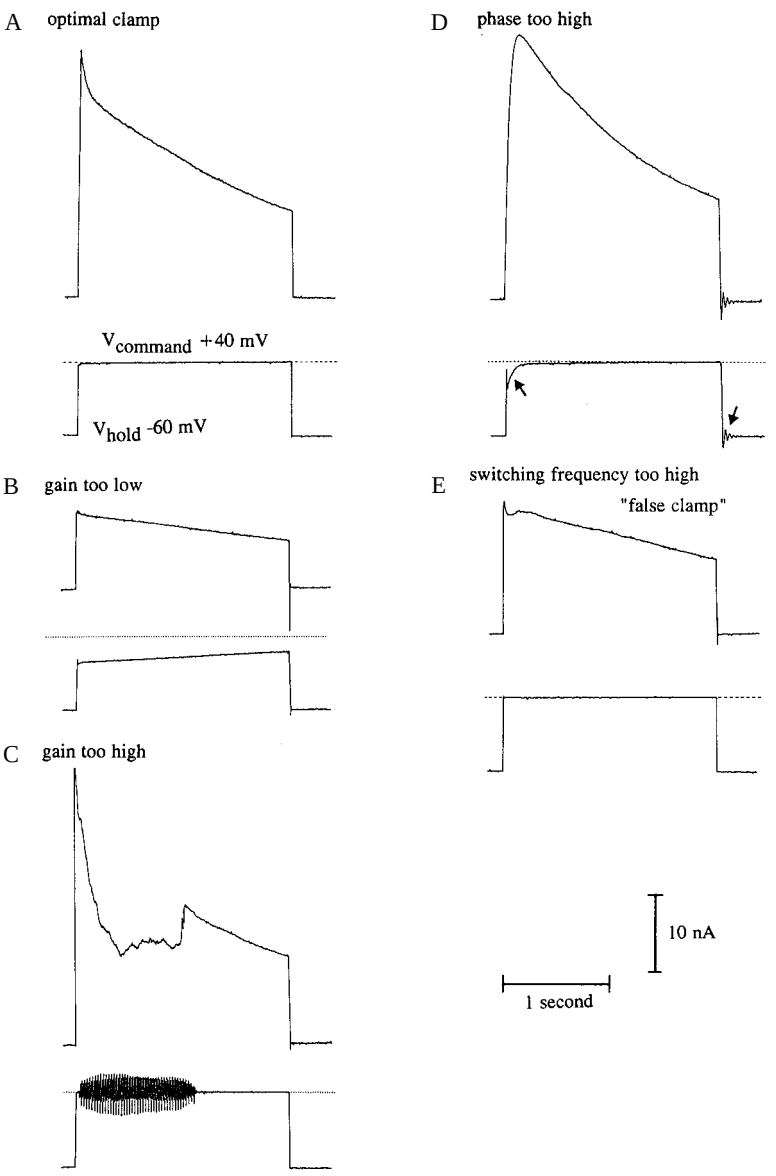

Fig. 6. Examples of correctly (A) and incorrectly (B-E) set-up SEVC implemented with an Axoclamp 2A instrument. Voltage dependent potassium currents were recorded from a neuroblastoma × glioma hybrid cell (NG108-15). Currents (upper traces) were evoked by 100 mV voltage commands from –60 mV for 2s (lower traces). Whole cell variant of the patch-clamp technique was employed; series resistance 8 MΩ, headstage gain X1. (A) Gain 7 nA/mv, phase-lag 0.2 ms and switching frequency 8 kHz. (B) As for A but gain reduced to 0.3 nA/mV. (C) As for A but gain increased 20 nA/mV. (D) As for A but phase-lag increased to 200 ms; note oscillations on the voltage trace (arrows). (E) As for A but switching frequency increased to 21 kHz; note that the voltage trace appears convincing but it is not reflecting the true membrane voltage owing to a systematic voltage error because of insufficient settling time for the voltage transient across the electrode resistance. Inspection of the headstage voltage output would reveal this error.

electrode clamp technique are usually for studying small cells, the large rejection rate on grounds of tip inadequacy (e.g. inability to impale cells) and a large consumption of micropipettes owing to fragility and propensity to tip blockage preclude complicated time-consuming procedures in the production of the electrodes. Often, attention to the recording situation and simple procedures such as coating with silicone oil or rubber will suffice to reduce electrode capacitance.

Impalement of small cells is often best accomplished by oscillating the capacity compensation circuit briefly ('zap', 'buzz' or 'tickle') or by applying a short ~20 ms intense current injection. Once a stable impalement has been achieved in the conventional bridge mode, the preamplifier may be switched to discontinuous current clamp mode and for further adjustment it becomes necessary to monitor the circuitry at three points to adjust the instrument's performance. Outputs from SH_1 (voltage) and SH_2 (current) are observed as is the voltage record just prior to SH_1, usually termed head stage out (HSO) or continuous voltage output. HSO indicates the signal V_m+V_e (Fig. 5) and with regular periodic current injection commands it is possible to observe the decay characteristics of V_e with a rapid oscilloscope sweep. Capacity compensation is adjusted to allow the fastest switching rate compatible with a full decay of V_e. Finally the SH_1 signal is monitored to check that V_m changes smoothly from rest with the onset of a current application and without showing an abrupt step, which could introduce an error in measuring V_m under conditions of current injection, similar to a series resistance error in a conventional clamp system. Errors of this sort (discussed in Chapter 1) are caused by a combination of inadequate capacity compensation (under or overadjustment) and an excessively fast cycling frequency so that the electrode transient contributes a significant component of the sampled voltage. Assuming that these checks have been made, V_c can be adjusted to the indicated resting value of V_m, S_2 can be set to voltage clamp mode and the cell brought under voltage control. Clamp gain is increased until the point of oscillation is almost reached and then reduced slightly. Clamp steps may now be imposed on the cell, as for the two electrode clamp. This may reveal a tendency for the clamp to oscillate owing to excessive loop gain. In this case, adjustment of the phase relation between voltage recording and current injection may negate this tendency. Most single electrode clamp devices possess an adjustment of this sort; in addition to introducing phase shifts, this control also changes the loop gain of the clamp in a frequency dependent manner.

The final parameter which *can* be changed but is often preset on single electrode clamp systems is the duty cycle. A duty cycle <50% allows more time for the V_e signal to decay but requires that a larger current be passed for the shorter period, thereby increasing the amplitude of V_e and also the chances of exceeding the current-passing capability of the (normally) high resistance electrode. Choice of duty cycle is obviously a compromise and the reader is referred to the more rigorous treatment of the subject by Finkel & Redman (1984).

As mentioned above the switching method can be employed with suction electrodes for the whole-cell patch clamp technique where series resistance errors can be minimised. However, from the discussion above it can be seen that inappropriate

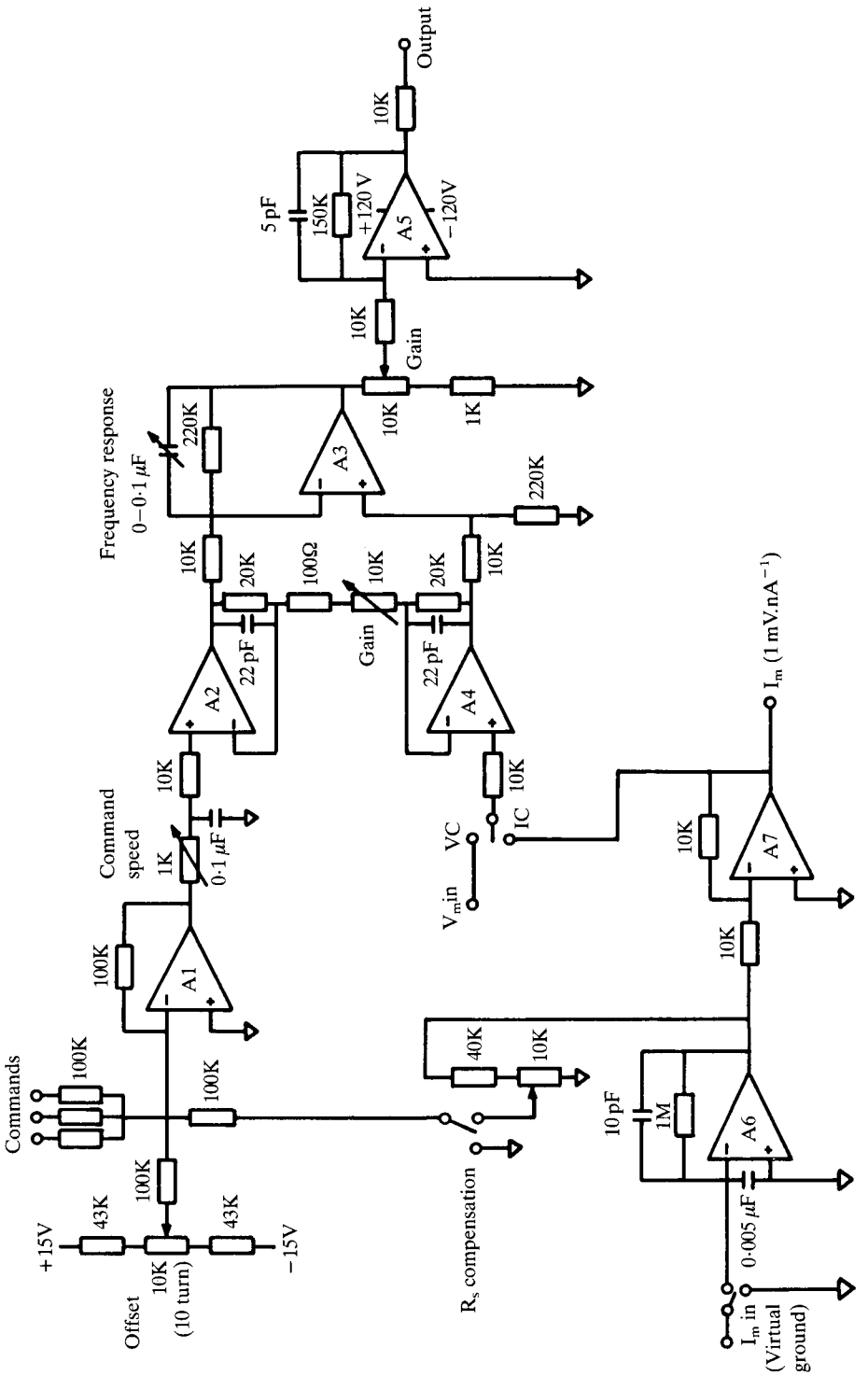

Fig. 7. Clamping amplifier circuit. Amplifiers A1-A4,A6, A7 are 3140E (RS Components) or 356 (National Semiconductor). A5 is a high-voltage operational amplifier - Teledyne Philbrick 1022 or Analog Devices 171J.

adjustment of the voltage clamp instrument can lead to errors that are equally bad. Figure 6 shows some results from a single electrode clamp experiment on a cultured neuroblastoma/glioma hybrid cell. Optimal clamp conditions reveal that a step depolarisation from resting potential (–60 mV) to +40 mV elicits a declining outward current. Inspection of the voltage trace reveals that within 10 ms the voltage attains the command potential. As with all modes of voltage clamp, insufficient loop gain causes a disparity between the potential command and that achieved (Fig. 6B). Because voltage is not constant, this situation, which *does* give a true measure of both current and voltage is not much better from the point of view of assessing membrane currents than an experiment with current clamp; if a full voltage command is assumed by the experimenter, the current measured will be underestimated. Conversely, with the gain too high, the clamp becomes unstable and both current and voltage traces display oscillations, which, in ignorance of the voltage trace, could lead an experimenter to misinterpret an actually smooth elicited current as a fluctuating one. At least, with the switching clamp method, attention to the voltage trace can alert the investigator to these insufficiencies; furthermore, the voltage indicated by the instrument reflects the true potential of the cell membrane subject to geometrical considerations pertaining to space-clamp. More hazardous are errors where too much lead or lag has been applied in the clamp timing; overshoot of the clamp potential with concomitant underestimation of command potential can ensue (Fig. 6D). Only very close attention to the voltage trace can signal this condition. Finally, when switching frequency is too high, the voltage samples the value of membrane potential together with an error due to insufficient decay of the voltage drop across the electrode (Fig. 6E): the command potential, while seeming to have been attained, in fact, falls short of the intended and consequently leads to an underestimate of the elicited current. The latter condition can be assessed and eliminated by careful inspection of the continuous headstage input voltage of the amplifier.

To conclude and recapitulate, the optimization of the single electrode clamp method can be effected by attending to the following points.

(i) Lowest electrode resistance that is feasible.

(ii) Minimize stray capacitance.

(iii) Fastest switching rate compatible with full decay of electrode voltage transient.

(iv) Maximum gain commensurate with clamp stability.

(v) Clamp noise can be reduced by filtering the output appropriately.

Appendix

A clamping amplifier for two microelectrode voltage clamp

Fig. 7 shows a clamping amplifier circuit designed for two microelectrode clamp of relatively large cells, such as molluscan neurones. The circuit is based on a conventional three operational amplifier differential amplifier (A2, A3, A4), with the

addition of a high voltage output stage (A5) that enables the amplifier to pass the high currents which may be required to clamp large cells. The frequency response of the amplifier may be altered by means of an 11-way switch which varies the feedback capacitor in the range below 0.1 μF.

Command pulses are fed in through a summing amplifier (A1) and are then passes through a variable low-pass filter so that their rise time may be adjusted. Membrane current is measured using a virtual-ground circuit (A6), while A7 inverts the current signal to the normal convention. The amplifier may be used to clamp membrane voltage (VC mode) or membrane current (IC mode). The membrane potential signal (V_m in) is derived from a differential preamplifier with a gain of 10. Compensation for series resistance (R_s compensation) is achieved by feeding a proportion of the membrane current signal to the summing point of amplifier A1.

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