

# COMPUTED MEMBRANE CURRENTS IN CARDIAC PURKINJE FIBERS DURING VOLTAGE CLAMPS

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**ABSTRACT** Recent measurements have indicated that some of the cardiac cell electrical capacitance is in series with a resistance. The computations of currents in a voltage clamp presented below show that, in this case, there is a danger that capacitive transient currents recorded during voltage clamp experiments may be confused with currents arising through rapid active membrane conductance changes. Secondly, a voltage clamp technique aimed at avoiding capacitive transients, namely the linear or ramp clamp, has recently been introduced. An attempt has been made here to evaluate the usefulness of ramp clamps in studying membrane electrical properties, by computing ramp clamp results and considering the difficulties in reconstructing the original model from these results. It is concluded that such a reconstruction is not feasible.

## INTRODUCTION

The usual method of studying the electrical properties of the Purkinje fiber membrane has been the application of rectangular voltage clamps to a portion of Purkinje fiber short enough to be almost uniformly polarized by intracellularly-applied currents. By such experiments, direct data on both time- and voltage-dependence of most membrane ionic conductances can be obtained. However, the method has failed to resolve the early currents which give rise to the initial depolarization "spike" of each action potential, because the electrical capacitance of the cell cannot be charged sufficiently rapidly to a new potential: fast transient currents are over before control of membrane potential is effected. The resistance which limits the current charging part of the cell capacitance (Fozzard, 1966) adds significantly to this difficulty. In order to avoid the large initial current needed to charge the capacitance, linear changes of membrane potential with time (ramps) have been used instead (Dudel, Peper, Rüdél, and Trautwein, 1966); under such conditions,

the current needed to maintain any given rate of change of potential on the capacitance is constant (except when some of the capacitance is in series with a resistance: see below), so that other early transient currents will not be obscured. Each ramp voltage clamp gives rise to a current-voltage relation termed "quasi-instantaneous"; such current-voltage relations are obtained much more quickly than those from rectangular voltage clamps, so that effects of membrane deterioration are also minimized. However, there are some serious disadvantages to this method as well. The various voltage-dependent conductance changes which give rise to excitability have widely differing time constants. When a ramp voltage clamp is applied, at any particular potential the slowly-changing conductances are still at values governed entirely by previous potential levels, while the quickly-changing conductances and the electrochemical potential gradients (driving forces) are determined solely by the latest level. There are, therefore, unavoidable difficulties in interpreting the measurements in terms of the individual ionic currents.

In this study, the Hodgkin and Huxley (1952) equations as modified for cardiac muscle by Noble (1962) have been used to compute the currents flowing during both types of voltage clamp mentioned above, and with various amounts of resistance in series with some of the cell capacitance. (A discussion of some reasons for favoring this equivalent circuit rather than more complex arrangements of capacitances and resistances has been included in McAllister, 1968). To improve the comparison between these and experimentally-derived results, the size of the clamp current has been limited. Since the maximum voltage output of the amplifiers used in voltage clamping is typically  $\pm 100$  v and the resistance of the current-passing microelectrode is typically  $20\text{ M}\Omega$ , such a limit certainly applies in practice; this maximal current will be flowing whenever the actual membrane potential deviates

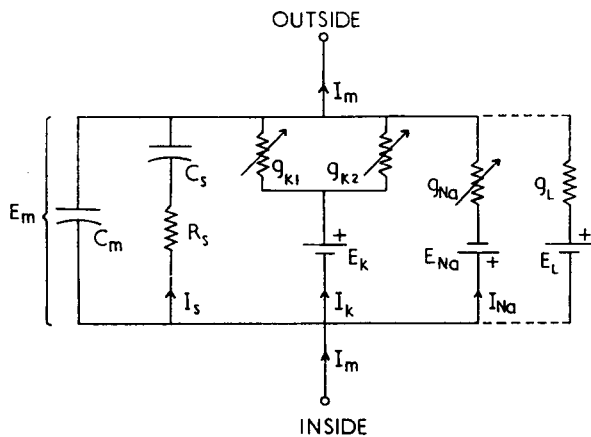


FIGURE 1 Equivalent electrical circuit representing the cardiac muscle membrane. "Leakage" currents would flow through the branch on the right; other elements of the circuit are described in the text.

from the desired clamp potential by approximately 1 mv or more, when typical amplifier gains are used.

## TERMINOLOGY

See Fig. 1. The total (low-frequency) capacitance of the Purkinje fiber will be called "cell capacitance"; that portion which has no resistance in series (and is most easily interpreted as the capacitance of the cell surface membrane) will be called "membrane capacitance"  $C_m$ ; and that portion which does have resistance in series will be denoted  $C_s$ . The "membrane potential"  $E_m$  is that existing across  $C_m$  at any time.

## METHODS

The equivalent circuit chosen to represent the Purkinje fiber membrane and the equations to be solved, have been discussed earlier (McAllister, 1968). The present calculations are simpler than those for an action potential, since the membrane potential  $E_m$  is no longer a free variable; with  $E_m$  and time  $t$  specified, the conductances and currents can be calculated directly.

A ramp voltage clamp was simulated by changing  $E_m$  in small steps and computing the values of the voltage- and time-dependent conductance parameters at the end of each short time interval; steps of 0.1 mv were usually employed, although there were no noticeable errors for steps of less than 0.3 mv. Thus from a given initial value  $m_0$ , the new value of  $m$  at the end of a time interval  $dt$  was given by

$$m = m_\infty - (m_\infty - m_0) \exp(-dt/\tau_m), \quad (1)$$

where  $m_\infty$  is the steady-state value of  $m$  at the new potential and  $\tau_m = (\alpha_m + \beta_m)^{-1}$  is computed from the values of  $\alpha_m$  and  $\beta_m$  at the new potential. The other conductance parameters  $m$  and  $h$  were treated similarly. (Noble's (1962) empirical equations for the  $\alpha$ 's,  $\beta$ 's, and instantaneous K conductance  $g_{K1}$  were used; all are functions of membrane potential only.) Multiplying each conductance by the driving potential for the related ion gave the current, e.g.,  $i_{Na} = g_{Na}(E_m - E_{Na})$ , and the sum of all these ionic currents was  $I_i$ . Finally, the constant current charging the membrane capacitance (of magnitude  $C_m dE_m/dt$ ) was added. If the total current then exceeded the maximum permissible value, that value was substituted and the computation for the step was repeated; but in this case, the change in membrane potential in response to a constant applied current  $I_m$  was computed, using the equation

$$C_m \frac{dE_m}{dt} + I_i = I_m. \quad (2)$$

In this way, periods of lost clamp control were included in the computation.

Rectangular voltage clamps were dealt with in the same way, except that (a) the current charging  $C_m$  was given by equation 2, because  $dE_m/dt$  was not constant during change of potential; and (b) once clamp control at the chosen potential was successfully established, it was assumed that the clamp was perfect thereafter. A consequence of (b) is that, since only time-dependent changes of an exponential nature could occur during clamp control, the time steps could be increased to speed computation.

In all cases it was assumed that, before initiation of the voltage clamp, the membrane had been at a potential of -80 mv long enough for all parameters to have attained their steady-

state values at that potential. For ramp voltage clamps of any specified slope, the current voltage relation was calculated for a depolarization from an initial level of  $-80$  mv up to  $+40$  mv, followed immediately by a repolarization to  $-100$  mv: thus the locus of membrane potential when plotted vs. time was triangular. For rectangular clamps, potential was clamped at the chosen level for 500 msec, and then currents were also calculated for a clamp back to the  $-80$  mv level. For either type of clamp, the clamp current at any moment was given by

$$- \text{gain} \times (E_m - E_c) \quad (3)$$

where "gain" is an over-all system gain defined by this expression, and  $E_c$  is the specified clamp potential; the clamp current was, however, limited to a magnitude of  $250 \mu\text{a}/\text{cm}^2$ . This maximum value of clamp current was chosen by assuming a maximum output of the clamp amplifier of  $\pm 100$  v, a microelectrode resistance of  $20 \text{ M}\Omega$ , and a cylindrical Purkinje fiber preparation measuring approximately 2 mm long and 0.3 mm in diameter exclusive of connective tissue sheath. The usual value of "gain" was  $1000 \mu\text{a}/\text{cm}^2 \cdot \text{mv}$ , so that maximal clamp current was reached if membrane potential differed from clamp potential by about 0.25 mv or more.

The effects of a resistance in series with part of the cell capacitance (a series-RC) were also computed. For a ramp voltage clamp, the current through the series-RC element is (from Laplace transform analysis)

$$i_s = C_s[1 - \exp(-t/R_s C_s)] \frac{dE_m}{dt} + i_0 \exp(-t/R_s C_s), \quad (4)$$

where  $i_0$  is the initial current (zero if the membrane potential was previously constant for some time); the currents were computed as for ramp clamps of membranes with no series-RC, and the appropriate value of  $i_s$  was added after each time step to get total current (which was not permitted to exceed a given maximum value, as explained above). The relevant equations for rectangular voltage clamps are in McAllister (1968).

Programs were written in Algol for a KDF-9 digital computer (English Electric-Leo-Marconi Computers Ltd., Kidsgrove, Stoke-on-Trent, England), which has a  $6\text{-}\mu\text{sec}$  cycle time, and the store required did not exceed 7,000 48-bit words. Each ramp voltage clamp was computed in approximately 80 sec, while rectangular voltage clamps required varying times up to 10 min.

## RESULTS

### *Rectangular Voltage Clamps*

When a rectangular voltage clamp to a level between threshold and  $E_{\text{Na}}$  (sodium equilibrium potential) is initiated, the initial current is maximal and positive (outward) to charge the membrane capacitance to its new potential; as soon as the action potential spike-generating mechanism is activated, however, the large inward Na current causes the membrane potential to move rapidly to a positive value and consequently clamp current will quickly reverse, to become maximal and negative. Then, when the Na current inactivates sufficiently so that net membrane current is less than  $250 \mu\text{a}/\text{cm}^2$ , the clamp will become effective and the clamp current thereafter will accurately reflect the net ionic current across the membrane. In Fig. 2,

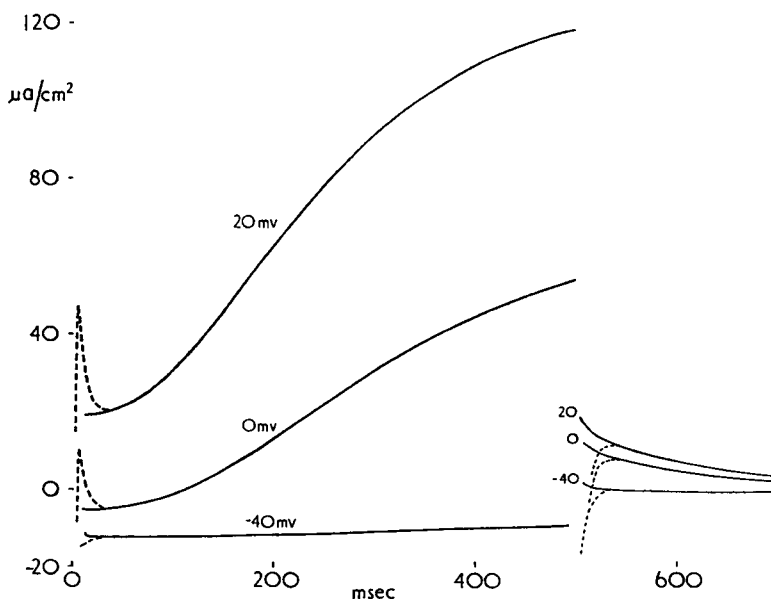


FIGURE 2 Computed membrane currents during rectangular voltage clamps to the potentials noted. Solid lines: current through a membrane with a pure capacitance of  $9.4 \mu\text{F}/\text{cm}^2$  and no series- $RC$  element. Dashed lines: alteration in current through the membrane if  $2.4 \mu\text{F}/\text{cm}^2$  is pure capacitance and  $7 \mu\text{F}/\text{cm}^2$  is in series with a resistance of  $1000 \Omega \cdot \text{cm}^2$ .

the very large transient currents which flow before the clamp is fully established have been omitted for clarity, while the portions likely to confuse experimentally-obtained records remain. The solid curves show the currents required according to Noble's (1962) model. It was found that in all cases, 5–7 msec are needed to establish the clamp, after which time no fast transient currents occur. Similarly, following repolarization back to  $-80 \text{ mv}$ , after approximately 5–7 msec the clamp becomes effective.

The dashed curves in Fig. 2 represent the currents required to clamp another membrane identical in all respects but one:  $7 \mu\text{F}/\text{cm}^2$  of the cell capacitance is in series with a resistance of  $1000 \Omega \cdot \text{cm}^2$ , while  $2.4 \mu\text{F}/\text{cm}^2$  has no resistance in series. This configuration is like that found by Fozzard (1966), except that the series resistance  $R_s$  is larger. Now the current early in the clamp is very different; following the maximal negative current which attempts to control the active sodium current, there is an additional transient current lasting until 30–40 msec after the beginning of the depolarization and, depending on the clamp potential level, either negative or positive. It becomes more positive as the depolarization is increased, and could possibly be mistaken for another active ionic current with a definite threshold. On clamping back to the initial potential of  $-80 \text{ mv}$  long-lasting transient currents again appear, and these are always negative. As they decay to zero, the clamp current becomes

more positive, then begins slowly to decline once more towards zero; the maximum outward current is reached 30–40 msec after initiation of the repolarization.

The essential point about these currents is their dependence *only* on the series- $RC$  element of the membrane. The potential on the capacitance  $C_s$  which is in series with  $R_s$  cannot be changed rapidly; it can be seen from Fig. 2 that its potential is slightly more positive than  $-40$  mv after the “spike” of sodium current has ended. (The spike referred to when discussing voltage clamp records is the current transient required to establish membrane potential at the new level.) The difference between the dashed and solid curves in Fig. 2 represents the current being drawn by the series- $RC$  element while  $C_s$  is charged to the clamp potential. For a clamp to  $-40$  mv,  $C_s$  must be discharged slightly (negative current) after the spike, while for clamps to 0 and  $+20$  mv, a large positive current is required for a few msec to charge  $C_s$  to the more positive potentials. Similarly, on repolarization the initial maximal negative current recharges the pure membrane capacitance  $C_m$  to  $-80$  mv, but a continued inward current is required for another 40–50 msec to recharge  $C_s$  to the same level. The duration of the transients in both cases will depend on the product  $R_s C_s$ .

While the series- $RC$  element can have a large effect on voltage clamp results, the

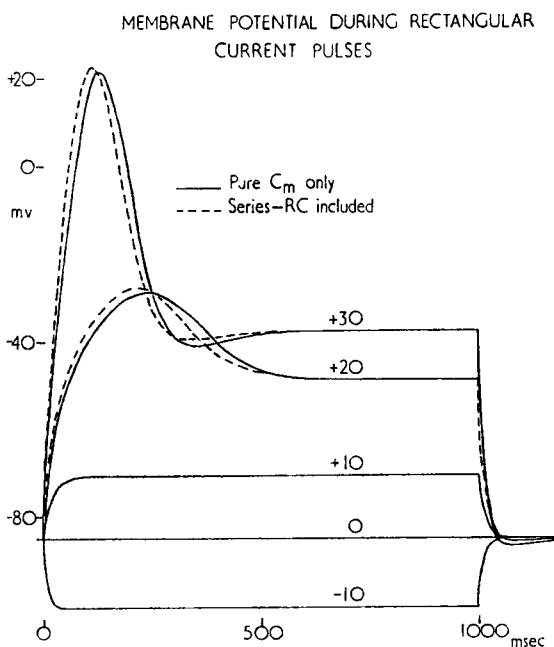


FIGURE 3 Alteration in membrane potential response to constant currents when a series- $RC$  is included in the membrane; values of capacitance and series resistance as in Fig. 2. The number above each gives the applied current in  $\mu\text{a}/\text{cm}^2$ .

responses to constant-current stimulation (as was used to investigate membrane properties previous to the advent of voltage clamp techniques) are not greatly altered; Fig. 3 illustrates the change caused by the same series- $RC$  element. For these computations, which are for comparison with experimental results in Na-free choline solutions, the sodium conductance has been set at 0 and therefore the membrane response to the applied current is very slow: no active depolarizing current is excited. The choline is assumed to "leak" sufficiently to give rise to a stable resting potential of  $-85$  mv; the equilibrium potential for the choline was taken to be  $+40$  mv, and the leakage conductance  $0.132$  mmho/cm<sup>2</sup>.

### *Ramp Voltage Clamps*

The "quasi-instantaneous" current-voltage relations obtained from depolarizations followed immediately by repolarizations at rates of  $0.01$  to  $10$  v/sec, for the membrane without series- $RC$  discussed above, are shown in Fig. 4. The constant current required to continually change the charge on the membrane capacitance (magnitude  $= C_m dE_m/dt$ ) has been omitted for the sake of clarity; it is positive during depolarization and negative during repolarization. It has been assumed in this case that the current required was at no time beyond the capacity of the amplifiers.

The large negative "hump" which begins to appear at  $-60$  mv for depolarization at  $0.3$  v/sec is due to the active sodium current which would normally cause the spike of the action potential. Its increase in size with increasing rate of depolarization is to be expected, since the conductance parameters are farthest from their end-values when the potential is changing most rapidly, and therefore they also change faster; since  $g_{Na}$  has a third-power dependence on the more rapidly changing activation parameter  $m$ , the latter's effects temporarily swamp the inactivating effect of  $h$ . The peak of the inward current occurs at more positive potentials with increasing rates of depolarization for similar reasons:  $h$  lags further behind during a more rapidly changing potential, so inactivation sets in later. The repolarization current-voltage relations are much more similar to each other, since at rates of  $1$  v/sec and over the fast conductance changes have been completed before the repolarization begins (although this is not true for rates greater than  $10$  v/sec, in which case a large negative Na current remains during some or all of the repolarization).

At rates greater than about  $1$  v/sec, the amount of delayed potassium conductance  $g_{K2}$  which has been activated when repolarization begins is very small, corresponding to the steady-state value at a membrane potential not far above resting level; as a result it continues to increase during part of the repolarization, and so the lower parts of the repolarizing current-voltage relation bend to the right above the resting level ( $-80$  mv). At rates slower than  $1$  v/sec, more  $g_{K2}$  is activated by the time repolarization begins, so the tops of the current-voltage relations are displaced to the right; but most of this has decayed again by the time  $E_m$  is down to  $-50$  mv, so the relatively greater Na current causes a shift to the left at the lower potentials.

The current-voltage relation as measured by very long rectangular current pulses is almost the same as that computed for the slowest rate in Fig. 4, but with no hysteresis. Even at a linear rate of change of up to 1 mv/sec, the conductance parameters all maintain virtually their steady-state values, so the current-voltage relation is midway between the two curves shown for the 0.01 v/sec rate.

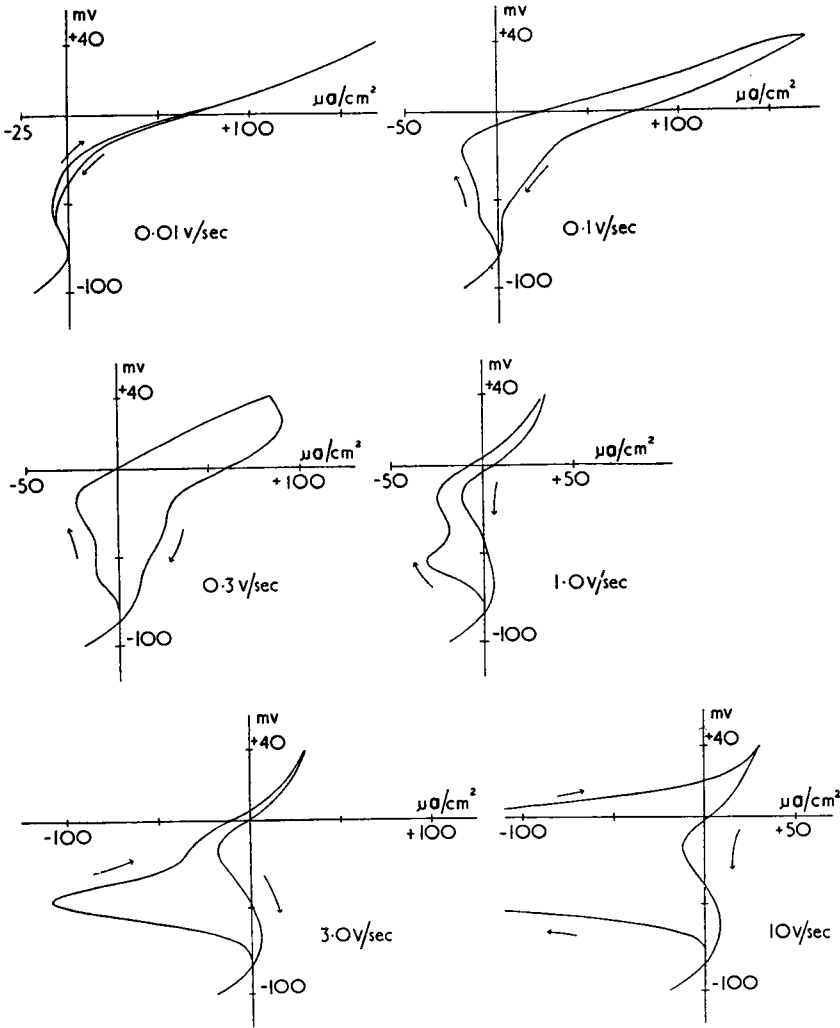


FIGURE 4 Computed current-voltage relations for ramp voltage clamps from  $-80$  to  $+40$  mv, then back to  $-100$  mv. All variables were initially at their steady-state values for  $-80$  mv. The rate of change of membrane potential is given in v/sec beside each curve. Arrows indicate the direction of change of membrane potential. Note the development of negative (inward) sodium current with increasing rate in the depolarizing relations, and its absence in the repolarizing ones. Constant capacitive currents have been removed.



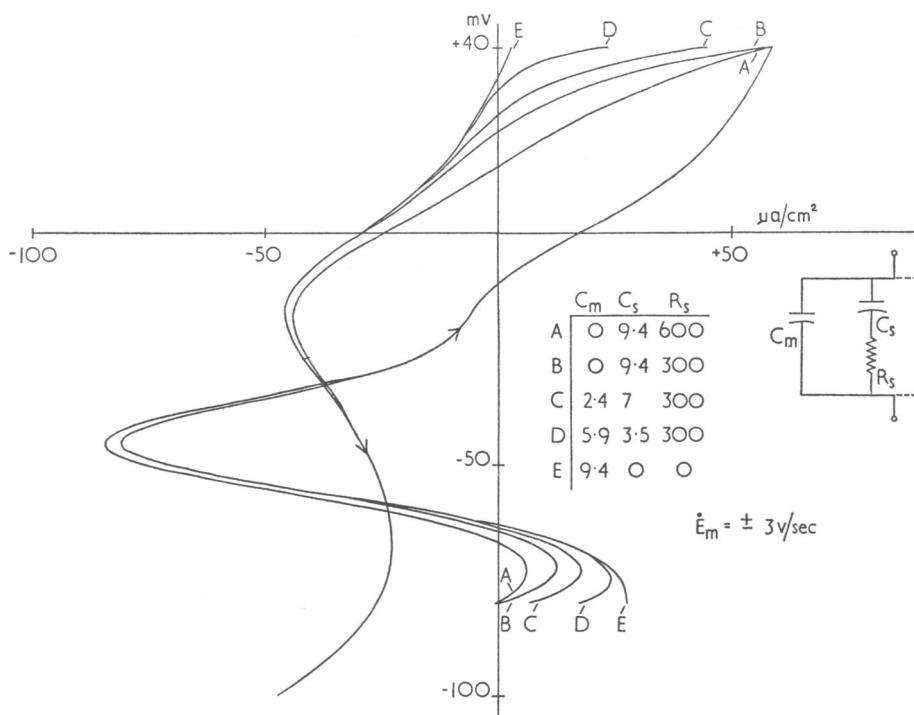


FIGURE 5 Computed effects of a series-RC element in the membrane on the current-voltage relations which arise during ramp voltage clamps. Rate of change of membrane potential is  $\pm 3$  v/sec. The values of  $C_m$ ,  $C_s$ , and  $R_s$  for each curve are indicated in the table above. Constant capacitive currents have been included; without these, curve E would coincide with the 3 v/sec curve in Fig. 4.

Fig. 5 shows the effects of a series-RC component in the membrane. The rate of change of membrane potential is 3 v/sec in every case, and the total capacitance of  $9.4 \mu\text{F}/\text{cm}^2$  is apportioned in various ways; curve C is for the values obtained by Fozzard (1966) for the Purkinje fiber. The series-RC gives rise to a transient and a constant current which both superimpose on the current-voltage relation for a membrane with no series-RC, curve E. The duration of the transient is determined by the product  $R_s C_s$ ; the magnitude of the constant shift is  $C_s dE_m/dt$ . Thus, doubling  $R_s$  while keeping  $C_s$  constant only increases the duration of the transient, while doubling  $C_s$  and keeping  $R_s$  constant causes the same increase in transient duration and also doubles the constant shift. This leads to a quick way of estimating  $C_s$  and  $R_s$  during experiments, as described in the Discussion section.

Fig. 6, top, shows two of the action potentials computed from the same equations used previously (McAllister, 1968); they differ substantially, one having a very large pre-plateau notch. Below these are corresponding current-voltage relations for rates of depolarization of 3 and 0.1 v/sec.

At 0.1 v/sec the results for the two cases are indistinguishable and are the same

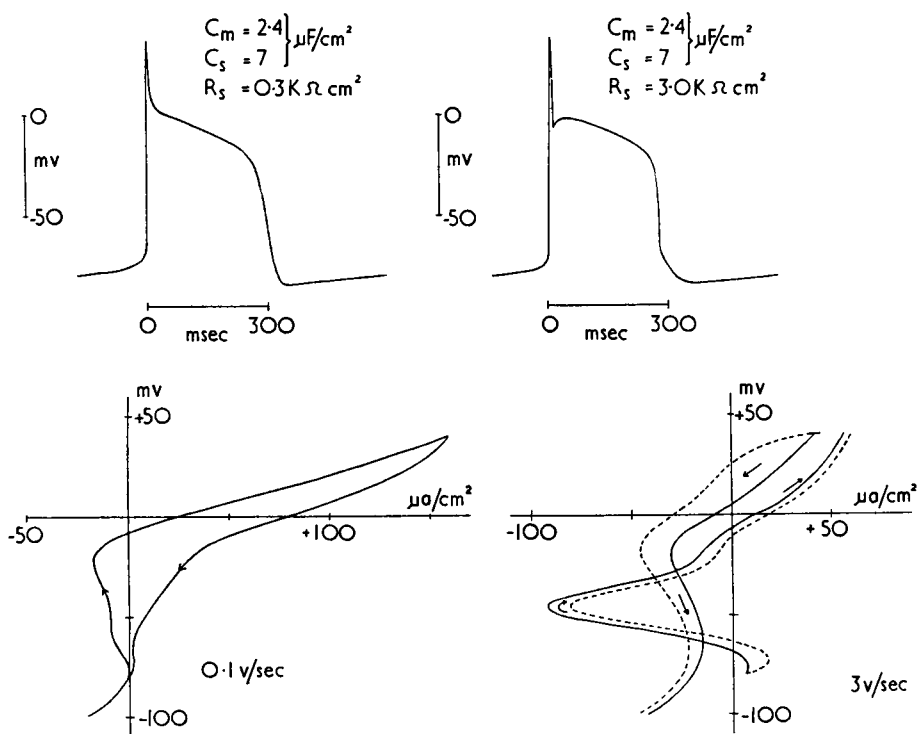


FIGURE 6 Computed current-voltage relations for ramp voltage clamps at 0.1 and 3 v/sec, for membranes giving rise to action potentials differing greatly in shape. The two current-voltage relations coincide at 0.1 v/sec. The dashed curve indicates  $R_s = 0.3$  and the solid one  $R_s = 3 \text{ k}\Omega\text{-cm}^2$ . Constant capacitive currents are included.

as for a membrane lacking a series-RC element (Fig. 4). At 3 v/sec, the membrane giving rise to a notched action potential passes less outward current and more inward current on depolarization and vice versa on repolarization; but the current-voltage relations are basically so similar that the difference in action potentials would probably not be suspected from this information alone.

Finally, Fig. 7 shows the type of error introduced into the current-voltage relations when the clamp current is insufficient to control the membrane potential part of the time. The inset shows the computed course of the membrane potential, for an attempted ramp voltage clamp depolarization at 10 v/sec. The dashed current-voltage relation is the one which should be obtained under continuous clamp control, while the solid one is that which would be recorded if the clamp current were limited to a magnitude of  $250 \mu\text{A}/\text{cm}^2$ . In the latter case, in the  $-50$  to  $0$  mV range membrane potential is actually at much more positive values than desired; as a result, the time- and voltage-dependent conductance parameters change more rapidly and toward different end-values than if the clamp were perfect. In particular, the Na-activation parameter  $m$  reaches a higher value and does so more rapidly, so that

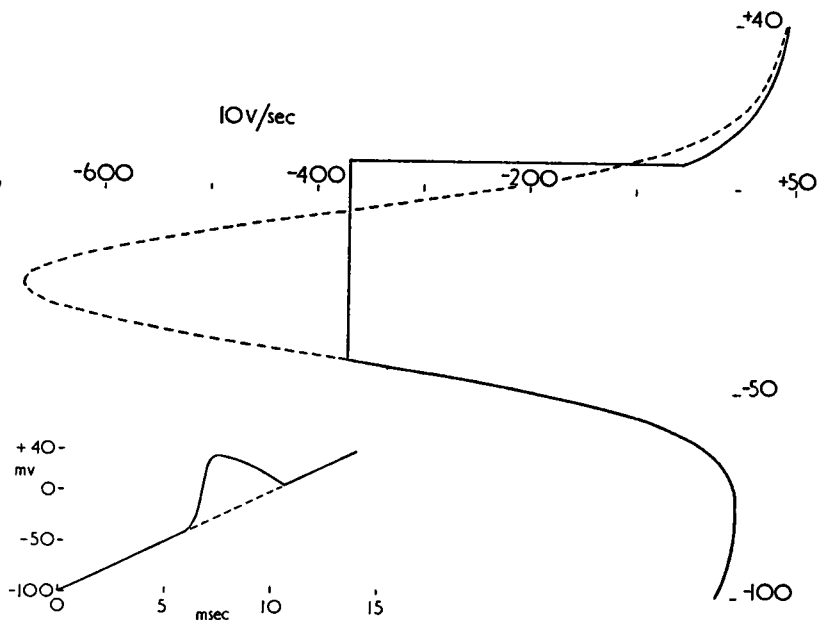


FIGURE 7 Currents through a membrane with  $C_m = 12 \mu\text{F}/\text{cm}^2$  and no series- $RC$  during ramp voltage clamp depolarizations from  $-100 \text{ mV}$  at  $10 \text{ v/sec}$ . Dashed curve: perfect clamp at all times. Solid curve: maximum clamp current limited to  $\pm 250 \mu\text{A}/\text{cm}^2$ . The  $+120 \mu\text{A}/\text{cm}^2$  artifact of the linear depolarization has been subtracted as in Fig. 3, so the apparent maximum is  $-370 \mu\text{A}/\text{cm}^2$ ; the current shown at each potential is therefore that which would exist if the depolarization were stopped at that potential.

the outward clamp current required is larger and longer-lasting; and the Na-inactivation parameter  $h$  finally reaches a lower value, so that when clamp control is regained (i.e., when the linearly-increasing reference potential reaches the actual membrane potential level) the current is less than at a corresponding potential level during a perfect clamp. The amount of error in any particular current-voltage relation will depend critically on both rate of depolarization and magnitude of maximum clamp current.

## DISCUSSION

The foregoing results are from computations for a membrane model which was not entirely based on experimental evidence: assumptions regarding the activation of the Na-carrying system and the voltage- and time-dependence of the K system were made (Noble, 1962). More recent work has provided much more information on the K system (McAllister and Noble, 1966, 1967; Noble and Tsien, 1968, 1969 *a*, 1969 *b*; etc.) but not much more is known about the Na system because, due to its fast time course, the characteristics have not been resolved by rectangular voltage clamp analysis.

The most interesting results of the Rectangular Voltage Clamps section concern the transient currents introduced into rectangular voltage clamp records by a series- $RC$  membrane element. These transients are unaffected by changes in the  $K$  system characteristics, since they decay before time-dependent  $K$  currents become significant. They would, therefore, not be appreciably different if more recent cardiac membrane models were used. Transient initial outward currents on depolarization, similar to those computed here, have already been noted in voltage clamp experiments (McAllister and Noble, 1966, Fig. 8 and associated text). Furthermore, the delayed rise to peak value of the outward current after repolarization, also illustrated in that paper, closely resembled the dashed curves in Fig. 2. The following points will show that careful observation of early transient currents in rectangular voltage clamp experiments is required if capacitive currents are not to be confused with time- and voltage-dependent ionic currents:

(a) The current charging  $C_s$  is voltage-dependent, since  $C_s$  will be charged to about the same potential during each "spike," but the subsequent current (i.e., after a true voltage clamp has been established) will be directly related to the clamp level. Therefore the degree of transient current "activation" is voltage-dependent.

(b) The current charging  $C_s$  will decline as  $C_s$  charges to the clamp potential; its magnitude, and therefore the time required to decay to any given level, will depend on the initial difference between  $E_c$  and the potential on  $C_s$  and also on the magnitude of  $R_s$ . There is therefore a time- and voltage-dependent transient current "deactivation."

(c) The magnitude of the current on repolarization will depend on the length of the previous depolarization as well as its level, unless  $C_s$  has completely charged to the clamp potential. It will also, of course, depend on the repolarization potential level and  $R_s$ .

The above analogy between passive capacitive transient currents and active ionic currents obeying Hodgkin-Huxley-type kinetics has been deliberately drawn to emphasize the tempting but mistaken analysis which could be applied in the absence of complete information on the membrane equivalent electrical circuit. Indeed, the only serious study of the complex membrane impedance of the Purkinje fiber published to date (Fozzard, 1966) has provided numerical results which suggest that there is in fact a real danger in ignoring the capacitive transients, if membrane currents during the first 50 msec of a clamp are being studied.

The results described in the Ramp Voltage Clamps section indicate that the hysteresis in current-voltage relations described by Trautwein, Dudel, and Peper (1965) can be explained to a large degree on the basis of present knowledge of the cardiac membrane. Fig. 4 shows that hysteresis occurs even for ramp voltage clamps at 0.01 v/sec, and the loop widens and shifts to the left when rate is increased just as in the results of Trautwein et al. Hysteresis occurs because the excitatory  $Na$  current is both voltage- and time-dependent. Its maximum occurs near  $-50$  mv during linear depolarization but it is almost completely inactivated by the time repolarization

begins unless the rate of potential change is faster than 10 v/sec. In addition,  $g_{K2}$  rises throughout the clamp, so that slower rates of change produce a shift of the top of the hysteresis loop toward the right. If Na-free solutions can be assumed to change  $E_{Na}$  to about  $-90$  mv (Dudel, Peper, Rüdell, and Trautwein, 1967), then the positions of the depolarizing and repolarizing current-voltage relations reverse in the computed case as in their experiments. This is very marked if  $g_{Na}$  is assumed unchanged by  $[Na]_0$  changes, and still occurs, though greatly diminished, even if slope conductance at  $E_{Na}$  is reduced to that predicted by the constant-field relation (which is probably an excessive reduction in this case) and Na currents at all potentials are reduced proportionately.

Inclusion in the mathematical model of recent data on time- and voltage-dependent K-conductance changes would be expected to alter the configuration of the hysteresis loops computed for slow ramp voltage clamps, but not appreciably affect the shape of those computed for faster ramp clamps (since then practically all the hysteresis is a consequence of  $g_{Na}$  changes only). Any accumulation of potassium ions outside the membrane (McAllister and Noble, 1966) during the long periods of polarization for slow clamps might affect the experimentally-observed relations, but no allowance has been made for this in the computations. It would cause a gradual reduction in the outward current required at any given potential level above  $E_K$  since the latter would become less negative, etc.

Because of the strong voltage- and time-dependence of the Na and K conductances, it does not seem a profitable avenue of approach to try to relate ramp voltage clamp results to the currents flowing during an action potential. However, Dudel et al. (1966) have used a fast ramp clamp to study the rapid conductance changes occurring during the AP spike; these currents are usually obscured by capacitive transients during rectangular clamps, as described above, but during ramp clamps the capacitive current is probably a constant plus a decaying transient and therefore presents less of a hindrance to analysis. They found a non-sodium current-voltage relation by locating  $E_{Na}$  for different extracellular Na concentrations; by subtraction they then plotted  $g_{Na}$  against membrane potential and found its magnitude varying greatly in proportion to rate of change of potential. They were unable to decide whether Na permeability differed substantially from that in other tissues in its dependence on  $E_m$  or whether the Na-inactivation was very fast (1–2 msec). The computed current-voltage relations of Fig. 4 are similar to their recorded ones in most respects, and they even include the second, smaller negative current "hump" near 0 mv and the shift in the depolarizing direction of the negative current maximum with increasing rate of change of  $E_m$ . These computations thus show that the sodium inactivation characteristics as measured by Weidmann (1955) and the activation characteristics formulated by Noble (1962) are adequate to reproduce most of the experimental observations obtained to date under ramp voltage clamp conditions. The equations in their present form do not, however, account for the unidentified large negative non-Na current seen at high rates (1–10

v/sec) by Dudel et al. (1966), or the dynamic positive component identified as chloride current by the same authors (1967). It is doubtful if ramp clamps will be of much use in measuring the exact time and voltage dependence of these components. They can, however, yield a time-integral of the active current that flows, and therefore show how the total amount is affected by experiments designed to alter it.

A further but unexploited use of the ramp voltage clamp techniques is in measuring  $C_m$ ,  $C_s$ , and  $R_s$ , since the constant and transient currents can be separated. When a set of experimental curves corresponding to those in the bottom right of Fig. 6 has been obtained for more than one rate of potential change, the circuit elements can be found. The instantaneous current change when rate  $S$  is suddenly reversed has a magnitude of  $2SC_m$ . The immediate change in the slope of the current-voltage relation on reversal is due to the transient current through  $R_s$  and this change has a magnitude given by  $-2/R_s$ . Finally, the shift along the current axis of some relatively rate-independent point of the current-voltage relation when different rates (e.g.,  $S_1$  and  $S_2$ ) are applied has a magnitude of  $(C_m + C_s)(S_1 - S_2)$  if the transient has died away (a useful reference point might be, for example, the current at  $-80$  mv during a repolarizing clamp when  $S$  is greater than 1 v/sec; see Fig. 4). This method may be applied to the solid curves in Fig. 3 of Dudel et al. (1966), and yields values roughly agreeing with those required in their model circuit to reproduce their experimental results. By comparing the measurements under conditions designed to alter parts of the cell morphology—e.g., separating the areas of close membrane apposition with hypertonic solutions (Barr, Dewey, and Berger, 1965) or rupturing a transverse tubular system (Gage and Eisenberg, 1967)—it may be possible to obtain some evidence as to where  $R_s$  and  $C_s$  actually reside.

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