VOLTAGE AND CURRENT CLAMP TRANSIENTS WITH MEMBRANE DIELECTRIC LOSS

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ABSTRACT Transient responses of a space-clamped squid axon membrane to step changes of voltage or current are often approximated by exponential functions of time, corresponding to a series resistance and a membrane capacity of 1.0 $\mu F/cm^2$. Curtis and Cole (1938, *J. Gen. Physiol.* **21**:757) found, however, that the membrane had a constant phase angle impedance $z = z_1(j\omega\tau)^{-\alpha}$, with a mean $\alpha = 0.85$. ($\alpha = 1.0$ for an ideal capacitor; $\alpha < 1.0$ may represent dielectric loss.) This result is supported by more recently published experimental data. For comparison with experiments, we have computed functions expressing voltage and current transients with constant phase angle capacitance, a parallel leakage conductance, and a series resistance, at nine values of α from 0.5 to 1.0. A series in powers of t^{α} provided a good approximation for short times; one in powers of $t^{-\alpha}$, for long times; for intermediate times, a rational approximation matching both series for a finite number of terms was used. These computations may help in determining experimental series resistances and parallel leakage conductances from membrane voltage or current clamp data.

INTRODUCTION

The sequence of events after the application of a step change of potential across a membrane in a voltage clamp is described as: (*a*) a capacitive transient of current as the change of potential is being established across the capacity of the membrane, followed by (*b*) the linear instantaneous leakage and other currents produced by appreciable ionic conductances, after which (*c*) the current changes according to the changes of the ionic conductances.

Where it has been necessary to estimate the instantaneous conductance it has become rather conventional to approximate the initial transient by an exponential decay with a time constant, $\tau = RC$, where C is the membrane capacity and R is the resistance in series with it. And indeed there has been a precedent from one of the first squid axon voltage clamp records in which the initial transient was shown as an exponential, on the phase plane for the membrane current (Cole, 1968, Fig. 3:18).

On the other hand, the first impedance measurements on the squid giant axon (Curtis and Cole, 1938) were interpreted as representing a lossy or dielectric membrane capacity with a constant phase angle impedance

$$z = z_1(j\omega\tau)^{-\alpha}, \qquad (1)$$

where the imaginary operator $j = (-1)^{1/2}$, $\omega = 2\pi f$ and f is the frequency. For $\alpha = 1$ the impedance is that of an ideal capacity but for the squid membrane α ranged from 0.7 to 0.95, with the average $\alpha = 0.85$ representing a phase angle $\phi = \alpha\pi/2 = 76°$. In the first of the famous voltage clamp series, Hodgkin et al. (1952, Fig. 16) showed a capacity transient which was distinctly different from a simple exponential. This they explained as an approximation to a dielectric capacity with $\phi = 80°$; but then they wisely ignored this discrepancy and only considered the membrane as a pure capacity of 1 $\mu F/cm^2$ throughout the rest of that series of papers as summarized in the last (Hodgkin and Huxley, 1952). Chandler and Taylor returned to membrane impedance measurements and from the single published figure (Taylor, 1965) the membrane impedance may be represented by Eq. 1 with $\alpha = 0.85$. In more recent measurements, Matsumoto et al. (1970) show impedance data which may be interpreted in terms of dielectric membrane capacities with α in the range 0.75–0.90.

The difference between the behaviors of a loss-free membrane capacity with $\phi = 90°$ and a dielectric capacity where $\phi = 75°$ may not be important for many purposes; but with increasingly fast electrode and electronics systems these differences may become significant. In voltage clamp, an exponential approximation will extrapolate to a higher value for the series resistance R at zero time than will a lossy transient which changes more rapidly at short times. As Taylor et al. (1960, p. 196) emphasized, the presence of an appreciable R distorts the potential applied to the membrane capacity and conductances from the ideal step and no method has been found to determine what the conductances would be after an ideal step. Various feedback circuits have been used to compensate for R but there should be an objective estimate of it. Conversely an exponential approximation can produce a low leakage resistance r because the lossy transient decays more slowly at long time than such an exponential. That is, a considerable part of the current remaining, after an exponential approximation has become negligible, may actually be mostly dielectric current rather than leakage (Moore et al., 1970). Thus, the determination of the subsequent changes of some or all of the individual ion conductances may be in error because the currents have not been measured from the true leakage current base line.

Another rather obvious way to measure R is with a step of current passed through the membrane in a current clamp. After an early jump, the potential across an ideal capacity rises linearly at first and the extrapolation back to zero time then produces the effective series resistance. This behavior was indeed found in the first current clamp (Cole, 1968, Figs. 3:7 and 3:8a). Recently the elimination of the initial jump has been used as a criterion for setting feedback circuits to compensate for the series resistance between the potential electrodes and the membrane capacity. If, however, the membrane has a lossy capacity, the early transient potential will not be strictly linear in time. In fact it will rise initially from the R value with an infinite slope. We have seen few records showing such nonlinear responses; but a report (C. M. Armstrong, personal communication) that only about two-thirds of the estimated R can be compensated without oscillation may be explained by a failure to observe such a dielectric response. Further, the accurate values of R, z_1, and r are important if only because no mechanisms for any of them are firmly established yet.

The most promising approach to these various interrelated problems is to determine if a dielectric impedance of the form $z_1(j\omega\tau)^{-\alpha}$ is a reasonable approximation to the observed transients over a considerable time range. It is thus necessary to compute the current transients under voltage clamp and potential transients under current clamp for dielectric membranes. To the extent that these computed transients agree with those observed, the calculations may be used to predict and correct for the membrane capacity components of current or potential where these cannot be observed directly. Specifically, the extrapolations $\tau \to 0$ should give R and $\tau \to \infty$ should give r.

In this paper, we give calculations of the transients for the circuit of Fig. 1 with a dielectric capacity. The process is similar in principle to that used by Cole and Cole (1942) to calculate the dielectric transients in which the resistors of Fig. 1 are replaced by capacitors. However, there is no comparison to be made with that work as to either the power of analysis or speed of computation now available. A preliminary report on this work has been made (Cole and FitzHugh, 1971).

MATHEMATICAL ANALYSIS

In principle, there is a choice of the fundamental approach to the computations. Approximations to impedances of the form $(j\omega)^{-\alpha}$ can be designed and constructed with lumped elements so that analogue solutions are possible (Lerner, 1963; Carlson and Halijak, 1964; Roy and Shenoi, 1966; Barnes, 1967). However, there are the difficulties of scaling to keep within the useful range of all the circuits, and of estimating the approximation errors in the usable range and the inherent limits of accuracy. Without assaying the various factors it seemed preferable to rely again upon the more familiar and powerful digital computer facilities which were available.

Voltage Clamp

The transient current in response to a step change of potential is calculated from the admittance $Y(p)$ of the circuit of Fig. 1. R and r are the series and parallel resistances. The impedance z and the admittance y of the constant phase angle capacitance are written:

$$z = z_1(\tau p)^{-\alpha}, \; y = y_1(\tau p)^{\alpha}, \; 0 < \alpha \leqq 1. \qquad (2)$$

z_1, y_1, and τ are constants. p is the argument of the Laplace transform, which is used here in place of the Fourier transform with argument $j\omega$, usually used in circuit

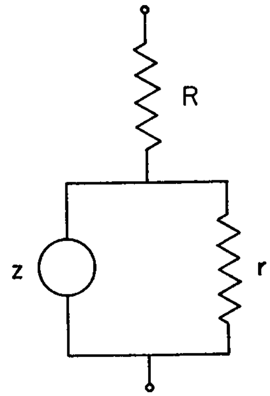

FIGURE 1 Equivalent circuit for squid axon membrane transients. z is the passive dielectric impedance given by Eq. 1, R is the series resistance including intra- and extracellular electrolytes, r represents the instantaneous, membrane leakage current.

analysis. The admittance of the whole circuit is:

$$Y(p) = \frac{1 + ry}{R + r + Rry}. \tag{3}$$

If the potential $v(t)$ takes the form of a step of amplitude $\bar{V}$, its Laplace transform is

$$\mathcal{L}[v(t)] = V(p) = \bar{V}/p. \tag{4}$$

The transform of the current $i(t)$ is

$$\begin{aligned} \mathcal{L}[i(t)] &= I(p), \\ &= Y(p)V(p), \\ &= \bar{V}Y(p)/p. \end{aligned} \tag{5}$$

For later use, define the constants:

$$\begin{aligned} G_1 &= 1/R, \qquad G_2 = 1/(R + r), \\ g &= G_1 - G_2 = r/[R(R + r)], \\ T_1 &= (gR^2y_1)^{1/\alpha}\tau. \end{aligned} \tag{6}$$

In order to calculate $i(t)$ as the inverse transform of $I(p)$, Eq. 3 is first converted to partial fraction form:

$$\begin{aligned} Y(p) &= \frac{1}{R + r}\left[1 + \frac{r}{R\left(1 + \frac{R + r}{Rry}\right)}\right] \\ &= G_2 + g/[1 + (T_1p)^{-\alpha}] \end{aligned} \tag{7}$$

From Eqs. 5 and 7 one can find $i(0)$ and $i(\infty)$ directly from limit theorems of Laplace transform theory (Doetsch, 1961, p. 207):

$$i(0) = \lim_{p\to\infty} pI(p) = \bar{V}G_1, \tag{8}$$

$$i(\infty) = \lim_{p\to 0} pI(p) = \bar{V}G_2. \tag{9}$$

Using Eq. 7 to rewrite Eq. 5:

$$\begin{aligned} I(p) &= \bar{V}\left[\frac{G_2}{p} + \frac{g}{p[1 + (T_1p)^{-\alpha}]}\right], \\ &= \bar{V}\,[(G_2/p + gT_1F(T_1p)], \end{aligned} \tag{10}$$

where

$$F(p) = \frac{1}{p(1 + p^{-\alpha})} = \mathcal{L}[f(t)]. \tag{11}$$

By the scaling rule for Laplace transforms (Doetsch, 1961, p. 227, no. 1):

$$F(T_1p) = \mathcal{L}[f(t/T_1)/T_1]. \tag{12}$$

The inverse transform of Eq. 10 is then

$$i(t) = \bar{V}[G_2 + gf(\bar{t})], \tag{13}$$

where

$$\bar{t} = t/T_1. \tag{14}$$

Here t is physical (dimensioned) time, while $\bar{t}$ is dimensionless (i.e., time measured in units of T_1). Below, in Table I and Figs. 2 and 3, computed values of the dimensionless function $f(\bar{t})$ vs. $\bar{t}$ are given, for different values of α. This distinction between dimensioned and dimensionless time should be kept in mind when comparing computed and experimental results. In the following derivation, however, where the distinction is unnecessary, we omit the bar over the t, to simplify the notation.

Express Eq. 11 as an infinite series:

$$F(p) = \sum_{k=0}^{\infty} (-1)^k p^{-1-\alpha k}. \tag{15}$$

This series, in descending powers of p, is absolutely convergent for $|p| > 1$. The series can be transformed term by term (Doetsch, 1961, p. 192; Doetsch, 1970, p. 208) to give:

$$f(t) = \sum_{k=0}^{\infty} \frac{(-1)^k t^{\alpha k}}{\Gamma(1 + \alpha k)}. \tag{16}$$

This series converges for all $t > 0$. It could in principle be used to calculate $i(t)$ (Eq. 13) to any accuracy for any value of t. In practice, however, if t is large, the number of terms needed becomes prohibitive. A different series expansion for $f(t)$ is available which can be used for large t.

Eq. 11 can be rewritten to give a series in ascending powers of p:

$$F(p) = \frac{1}{p}\left(1 - \frac{1}{1 + p^{\alpha}}\right),$$

$$= \sum_{k=1}^{\infty} (-1)^{k+1} p^{\alpha k - 1}. \qquad (17)$$

Leading terms of this series become infinite at $p = 0$, but elsewhere inside a neighborhood of the origin with radius 1, the series is absolutely convergent. From Eq. 17 is obtained the following asymptotic expansion for $f(t)$ as $t \rightarrow \infty$ (Doetsch, 1961, p. 213; Doetsch, 1970, p. 271):

$$f(t) \approx \sum_{k=1}^{\infty} \frac{(-1)^{k+1} t^{-\alpha k}}{\Gamma(1 - \alpha k)}. \qquad (18)$$

An asymptotic expansion differs from a convergent series as follows. Let $f_K(t)$ denote the finite sum obtained by replacing the upper limit of summation in Eq. 16 by K. Then for any *fixed t*,

$$\lim_{K \to \infty} f_K(t) = f(t)$$

since the series is convergent. If $f_K(t)$ is defined similarly for the series in Eq. 18, however, one has only the weaker condition that for any *fixed K* (Doetsch, 1961, p. 209):

$$\lim_{t \to \infty} t^{\alpha K}[f(t) - f_K(t)] = 0.$$

That is, as t approaches infinity, the difference between $f(t)$ and its Kth partial sum approaches zero faster than the last term of the partial sum itself.

The convergent series can theoretically be evaluated to any degree of accuracy for every value of its argument, by simply taking enough terms of the series. The asymptotic expansion, on the other hand, attains a specified accuracy only if t approaches close enough to infinity. For smaller values of t it may not be possible to reach the same accuracy, no matter how many terms are taken.

Current Clamp

No new calculations are needed for the current clamp transients. From Eqs. 2, 3, the impedance of the circuit is

$$Z(p) = 1/Y(p) = \frac{R + r + Rry}{1 + ry};$$

$$= R + r - \frac{r}{1 + (1/ry)},$$

$$= R + r - \frac{r}{1 + (T_2p)^{-\alpha}},$$

where

$$T_2 = (ry_1)^{1/\alpha}\tau .$$

For a step current of amplitude $\bar{I}$,

$$I(p) = \bar{I}/p,$$

$$V(p) = Z(p)I(p),$$

$$= \bar{I}\left\{\frac{R + r}{p} - \frac{r}{p[1 + (T_2p)^{-\alpha}]}\right\},$$

$$= \bar{I}[(R + r)/p - rT_2F(T_2p)],$$

$$v(t) = \bar{I}[R + r - rf(\bar{t})],$$

where

$$\bar{t} = t/T_2 .$$

Since the current clamp transient $v(t)$ can also be expressed in terms of the function $f(\bar{t})$, a separate computation is not required.

Special Cases

The function $f(t)$ can be found directly from tables of Laplace transforms for two values of α.
For $\alpha = 1$,

$$F(p) = 1/(p + 1),$$

$$f(t) = \exp(-t).$$

For $\alpha = \frac{1}{2}$,

$$F(p) = 1/[p + (p)^{1/2}],$$

$$f(t) = \exp(t) \operatorname{erfc}[(\bar{t})^{1/2}]$$

(Doetsch, 1961, p. 230, no. 35 and p. 241, no. 171).

The two series expansions can be used for computing $f(t)$ for small and large values of t, but are unsatisfactory for intermediate values of t. The convergent series 16 requires more and more terms as t increases, and a point is reached at which the accumulated roundoff error reduces the accuracy of the result. As mentioned above, the asymptotic series cannot be trusted for t too small (and it is not certain how small is too small). To alleviate this difficulty, it is better to use a rational approximation for $f(t)$ which agrees with both series up to a certain number of terms, at the two ends of the range of t, and moreover provides a smooth transition between these two series for intermediate values. (This rational approximation differs from the usual ones in the Padé table, [Wall, 1948; Kogbetlianz, 1960] which agree with only a *single* series up to a certain number of terms.) Define

$$x = t^{\alpha},$$

$$s_k = (-1)^k/\Gamma(1 + \alpha k),$$

where k is any integer, positive, negative, or zero. The series 16 and 18 can be rewritten:

$$S_C(x) = \sum_{k=0}^{\infty} s_k x^k,$$

$$S_A(x) = -\sum_{k=-\infty}^{-1} s_k x^k.$$

Approximate both series by a rational function of x, i.e. the ratio of two polynomials $A(x)/B(x)$, where

$$A(x) = \sum_{k=0}^{N} a_k x^k,$$

$$B(x) = 1 + \sum_{k=0}^{N} b_k x^{k+1}. \qquad (19)$$

Then one can write the approximation:

$$A(x) \doteq S_C(x)B(x) \doteq S_A(x)B(x). \qquad (20)$$

Here $S_C(x)B(x)$ contains powers of x from 0 to infinity; $S_A(x)B(x)$, from minus infinity to N. The overlap in these two ranges of powers is from 0 to N, which is also the range of powers for $A(x)$. The approximation will be assumed to be a strict equality for this range of powers; powers outside it are neglected. First, find the coefficients b_k by solving the resulting right-hand equation in Eq. 20:

$$\left(\sum_{k=-\infty}^{\infty} s_k x^k\right)\left(1 + \sum_{m=0}^{N} b_m x^{m+1}\right) = 0$$

$$\sum_{h=-\infty}^{\infty} \sum_{m=0}^{N} s_h b_m x^{h+m+1} = -\sum_{k=-\infty}^{\infty} s_k x^k. \qquad (21)$$

Now equate the coefficients of x^k on both sides of Eq. 21, for $k = 0, 1, \ldots, N$, ignoring other powers.

$$\sum_{m=0}^{N} s_{k-m-1} b_m = -s_k .$$

This set of N simultaneous equations in the b_k can be solved numerically. The a_k can then be found by either of the following equations, obtained by setting $A(x)$ equal to either of the other two expressions in Eq. 20, and equating coefficients as before.

$$a_k = s_k + \sum_{m=0}^{k-1} s_{k-m-1} b_m ,$$
$$= - \sum_{m=k}^{N} s_{k-m-1} b_m .$$

COMPUTATIONS

Programs to compute $f(t)$ were written in FORTRAN and SAIL (a version of ALGOL 60) for the PDP-10 time-sharing computer. First, the two series 16 and 18 were evaluated, cutting off the series when the last term used did not change the result to within a specified relative accuracy (usually 10^{-8}, but the cutoff point was rather insensitive to the accuracy used). The number of terms required increased greatly for values of t in the middle of the range (see below).

Computation time was reduced by assuming α to be rational, i.e. $\alpha = m/n$, where m and n are small integers (20 was the maximum value of n used). For the convergent series, Eq. 16 can be written

$$f(t) = E_\alpha(-t^\alpha), \tag{22}$$

where

$$E_\alpha(x) = \sum_{k=0}^{\infty} \frac{x^k}{\Gamma(1 + \alpha k)} . \tag{23}$$

$E_\alpha(x)$ is the Mittag-Leffler function (Sansone and Gerretsen, 1960, p. 345) used in the theory of functions of a complex variable. Separate the index k into two parts, $k = jn + r$, where r is the remainder obtained by dividing k by n. Then $\alpha k = \alpha r + \alpha jn = \alpha r + jm$, and Eq. 23 can be rewritten:

$$E_\alpha(x) = \sum_{j=0}^{\infty} x^{jn} \sum_{r=0}^{n-1} P_{jr} , \tag{24}$$

where

$$P_{jr} = \frac{x^r}{\Gamma(\alpha r + jm + 1)} . \tag{25}$$

Then by the recursion property of the gamma function [i.e., $\Gamma(z+1) = z\Gamma(z)$],

$$\Gamma(\alpha r + jm + 1) = (\alpha r + jm)(\alpha r + jm - 1)\dots$$
$$\dots[\alpha r + (j-1)m - 1]\Gamma[\alpha r + (j-1)m - 1], \tag{26}$$

$$P_{jr} = \frac{P_{j-1,r}}{(\alpha r + jm)\dots[\alpha r + (j-1)m - 1]}. \tag{27}$$

By assuming α rational, one needs to compute the gamma function a total of only n times, instead of once for each term in the series, a savings of computer time if many terms of the series are used. A similar trick is used with the asymptotic series 18.

For t in the middle of its range, so many terms of the series are needed (up to 85 terms of the convergent series at $t = 10$) that a large accumulated roundoff error is possible. Even using double precision throughout did not eliminate this difficulty. For $t = 20$, neither series could be used; either the value computed was obviously in error, or computation was stopped by overflow. The rational approximation $A(x)/B(x)$ was therefore used (Eq. 19). It agreed (as expected) with the two series at the ends of the range of t, and provided better values of $f(t)$ for the middle of the range. The degree N of $A(x)$ was increased until the value of $f(t)$ no longer changed (to within the desired accuracy). Near the ends of the range of t, $N = 10$ or even 5 gave accurate results. A maximum of $N = 20$ was used, except for $\alpha = 0.9$ and 0.95, where up to $N = 30$ was tried. These larger values of N again made large accumulated roundoff errors possible, and for $t = 10$, $\alpha = 0.9$ and 0.95, the last digit shown in Table I is in doubt.

Table I shows values of $f(t)$ [shown as $f(\bar{t})$, to emphasize that the argument is dimensionless time]. Values were computed for nine values of α between 0.5 and 0.95, using the rational approximation (for $\alpha = 1.0$, the exponential function was used, see previous section). Four significant figures in both $f(t)$ and $1 - f(t)$ are preserved, up to a maximum of seven decimal places. By the test of convergence with increasing N, these four significant figures are believed to be valid (except as mentioned above). Figs. 2 and 3 show $f(t)$ plotted in two different ways (see legends for explanation).

There is another possible source of error in the rational approximation. Examination of the values of $A(x)$ and $B(x)$ showed they changed sign together one or more times, for higher values of N. The roots of both polynomials were computed by a modified method of false position (Hamming, 1971, p. 47). Each pair of computed roots, one for $A(x)$ and one for $B(x)$, agreed to within a relative difference of between zero and $2 \cdot 10^{-5}$, with three-fourths of them being less than 10^{-7} apart. Since the intrinsic roundoff error of the PDP-10 computer due to word length is about 10^{-8}, these results strongly suggest that $A(x)$ and $B(x)$ in fact have common roots. Why this should be so (if it is so) is an interesting unanswered question.

If the roots do coincide at $x = x_1$, then there is a common factor $(x - x_1)$ in both polynomials which can be canceled out of the rational approximation. This was

TABLE I

$f(\bar{t})$ VS. $\bar{t}$ AND α

$\bar{t}$ \ α....	1.00	0.95	0.90	0.85	0.80	0.75	0.70	0.60	0.50
0.00000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000	1.0000000
0.00001	0.9999900	0.9999819	0.9999671	0.9999405	0.9998926	0.9998066	0.9996521	0.998882	0.996442
0.00002	0.9999800	0.9999650	0.9999386	0.9998928	0.9998131	0.9996747	0.9994349	0.998306	0.994974
0.00005	0.9999500	0.9999163	0.9998601	0.9997665	0.9996110	0.9993533	0.998927	0.997067	0.992071
0.00010	0.9999000	0.9998383	0.9997389	0.9995791	0.9993228	0.998913	0.998258	0.995559	0.98882
0.00020	0.9998000	0.9996876	0.9995128	0.9992415	0.998821	0.998172	0.997172	0.993280	0.98424
0.00050	0.9995001	0.9992541	0.998889	0.998348	0.997549	0.996370	0.994638	0.98840	0.97526
0.00100	0.9990005	0.998560	0.997928	0.997025	0.995737	0.993905	0.991309	0.98249	0.96529
0.00200	0.998002	0.997219	0.996137	0.994644	0.992591	0.98978	0.98593	0.97363	0.95147
0.00500	0.995012	0.993373	0.991212	0.98837	0.98465	0.97980	0.97351	0.95494	0.92496
0.01000	0.990050	0.98724	0.98367	0.97916	0.97347	0.96633	0.95743	0.93285	0.8965
0.02000	0.98020	0.97550	0.96976	0.96279	0.95435	0.94420	0.93207	0.90077	0.8585
0.05000	0.95123	0.94254	0.93250	0.92097	0.90782	0.8929	0.8762	0.8370	0.7904
0.10000	0.90484	0.8921	0.8781	0.8628	0.8461	0.8283	0.8092	0.7679	0.7236
0.20000	0.8187	0.8026	0.7858	0.7684	0.7506	0.7326	0.7144	0.6785	0.6438
0.50000	0.6065	0.5941	0.5826	0.5720	0.5623	0.5536	0.5458	0.5329	0.5232
1.00000	0.3679	0.3716	0.3761	0.3812	0.3869	0.3931	0.3996	0.4133	0.4276
2.00000	0.1353	0.1587	0.1811	0.2027	0.2235	0.2437	0.2632	0.3006	0.3362
5.00000	0.006738	0.02540	0.04522	0.6607	0.08783	0.1104	0.1336	0.1820	0.2323
10.00000	0.0000454	0.00761	0.01726	0.02903	0.04298	0.05910	0.07736	0.1201	0.1706
20.00000	0.0000000	0.003365	0.008037	0.01428	0.02238	0.03260	0.04520	0.7838	0.1232
50.00000	0.0000000	0.001309	0.003272	0.006106	0.01008	0.01550	0.02276	0.04450	0.07901
100.00000	0.0000000	0.0006621	0.001711	0.003304	0.005648	0.009012	0.01374	0.02908	0.05614
200.00000	0.0000000	0.0003388	0.0009056	0.001809	0.003200	0.005288	0.008355	0.01905	0.03980
500.00000	0.0000000	0.0001409	0.0003938	0.0008228	0.001523	0.002634	0.004358	0.01093	0.02521
1000.00000	0.0000000	0.0000727	0.0002104	0.0004550	0.0008715	0.001560	0.002672	0.007188	0.01783
2000.00000	0.0000000	0.0000376	0.0001126	0.0002519	0.0004995	0.009254	0.001641	0.004733	0.01261
5000.00000	0.0000000	0.0000157	0.0000493	0.0001155	0.0002396	0.0004647	0.0008624	0.002727	0.007978
10000.00000	0.0000000	0.0000081	0.0000264	0.0000640	0.0001375	0.0002761	0.0005305	0.001797	0.005642

tried, but did not improve the accuracy of $f(\bar{t})$ for $\bar{t} = 20$, according to the convergence test.

If, through errors of approximation, the actual roots of the polynomials used did not coincide exactly, then the rational approximation has a pole at the root of $B(x)$ and goes to infinity there, which could introduce a very large error in the immediate neighborhood of the root. However, even when values of $\bar{t}$ spaced logarithmically at 10 steps per decade were used,[1] no such error large enough to be detected in a plotted curve was evident. In conclusion, the rational approximation provides a satisfactory method for computing $f(t)$, and the possibilities of inaccuracy in the middle of the t range never become serious.

Two other methods for computing $f(t)$ were suggested to us using continued fractions or the integral formula for the Mittag-Leffler function (Sansone and Gerretson, 1960, p. 346; Erdelyi, 1955, p. 206). Attempts to use continued fractions constructed by Viskovatoff's procedure (Wall, 1948; Khovanskii, 1963) to the convergent series 16 were unsuccessful, because overflow occurred before convergence was reached. This approach was not pursued further but might be made to work.

[1] A table giving these results is available on request.

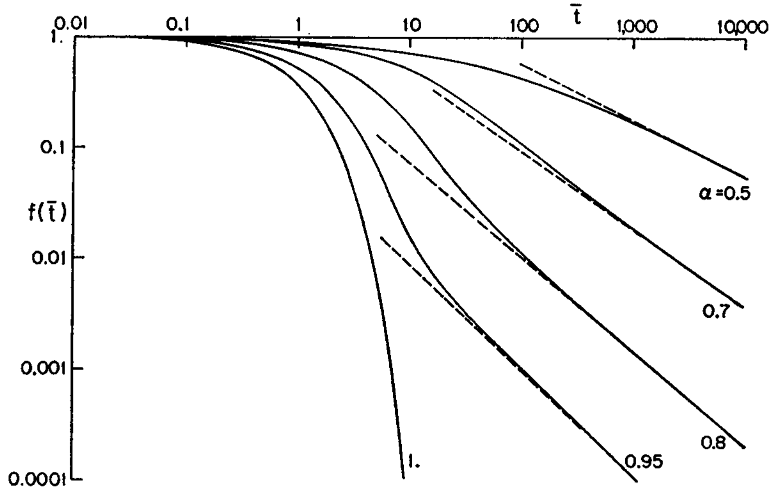

FIGURE 2 $f(\bar{t})$ vs. $\bar{t}$, log-log plot. Curves have been shifted horizontally to the right by the following amounts to separate them.

α	Shift in $\log_{10}$ units
0.5	2.0
0.7	1.2
0.85	0.6
0.95	0.2
1.0	0.0

Straight broken lines represent first term of asymptotic series (Eq. 18).

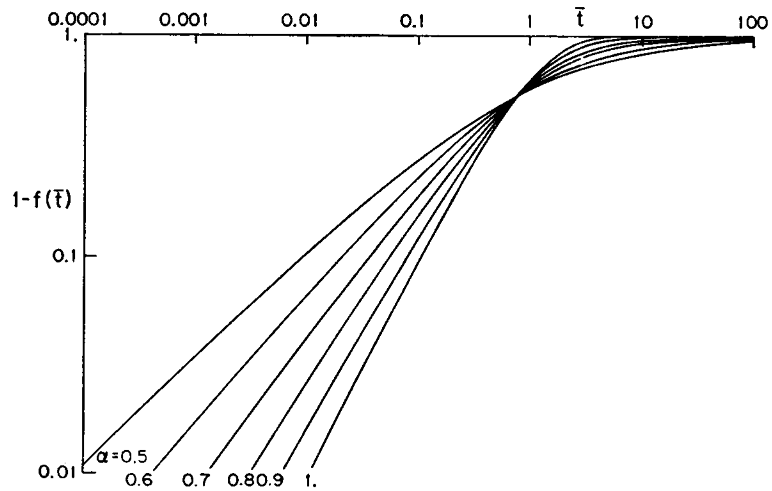

FIGURE 3 $1 - f(\bar{t})$ vs. $\bar{t}$, log-log plot. For small t, curves approach straight lines given by first term of convergent series (Eq. 16).

A few computations were made using the contour integral in the complex plane transformed to a pair of real integrals. The numerical integration was done with the differential equation solver in the mathematical modeling program MLAB (Knott and Reece, 1972; Knott and Shrager, 1972) on the PDP-10. Much more computer time was needed than for the rational approximation. The values obtained were:

α	$\bar{t}$	$f(\bar{t})$ from integral	$f(\bar{t})$ from rational approximation
0.8	1.	0.3869	0.3869
0.8	10.	0.04297	0.04298
0.9	10.	0.01724	0.01726
0.95	10.	0.00758	0.00761

The agreement, though not perfect, indicates that there are no gross errors in either method for $\bar{t}$ in its midrange.

Finally, $f(t)$ was computed by numerical inversion of the Laplace transform $F(p)$ (Eq. 11), using Stehfest's algorithm (Stehfest, 1970) in double precision. Stehfest recommends caution in using this algorithm on an unknown function and says that "the accuracy should be checked using other inversion techniques." Computations with his algorithm confirmed but did not improve those obtained with the rational approximation. Readers wanting to do this computation themselves might prefer to use Stehfest's algorithm (written in ALGOL 60) to save programming effort.

The rational approximation seems to provide best accuracy with economy of computer time. If more accuracy were required in the middle range of values of $\bar{t}$, the best procedure might be to repeat the rational approximation computations with double precision and higher degree and hope that coincident roots of the polynomials did not cause too much trouble.

APPLICATION

Without comparable high speed records of capacity transient currents under voltage clamp, we have confined our attention to those given by Hodgkin et al. (1952, Fig. 16) at 8°C for ±40 mV steps from the rest potential. The two records are different, with hyperpolarization giving a slightly higher value for α, but they have been averaged for the comparison with our calculation at $\alpha = 0.9$ as shown in Fig. 4. The authors remark that these records "... are roughly consistent with a constant phase angle of 80°, while those at higher temperatures require somewhat lower values." We regret that we do not know how this "rough" consistency was obtained! However, the agreement at the shortest times is closer than it should be. The delays of the clamp and current amplifiers appear as the displacement of the zero time abscissa for the computed curve as indicated on Fig. 4. Hodgkin et al. approximate these delays

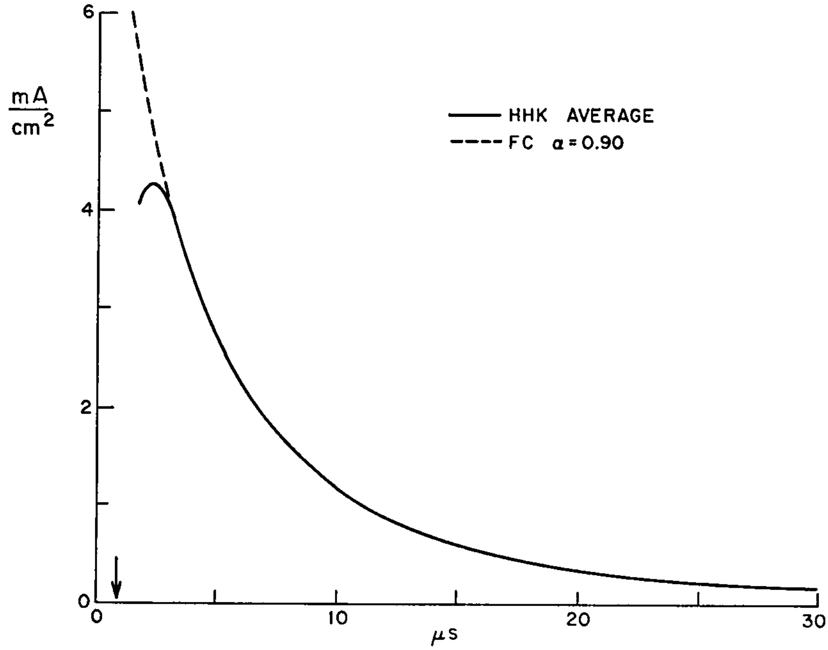

FIGURE 4 Comparison of the experimental and calculated charging currents in voltage clamps. HHK denotes the mean currents at ±40 mV from rest (Hodgkin et al., 1952). FC is the current as calculated for $\alpha = 0.9$, but the curve has been displaced to the right with its time origin indicated by the arrow, to provide an empirical correction for the delays in the voltage clamp pulse and the current amplifier response.

by a 1 μs exponential which should not allow coincidence of the curves to appear as early as shown.

A routine method has not been developed for comparing experimental voltage clamp curves with computed results; but a procedure we have used for fitting exponentials should be useful. In this, a slide would be made for each value of α with a dozen curves for $f(\bar{t})$ as a function of $\bar{t} = t/T$ for appropriate values of $\bar{t}$ from 0.2 to 5.0. By projecting one of these families of curves on an experimental curve with an autofocus enlarger it should be easily possible to interpolate between the pair of calculated curves which give the closest fit.

The early transient potentials under current clamps have had even less careful attention. The first records (Cole, 1968, Fig. 3:8a) were linear to within the resolution of the traces, as have been some more recent records. Only a few have been found with a definite curvature but a reasonable method for approximating them by the $1 - f(\bar{t})$ calculations has not been found. Also the corresponding voltage clamp transients have not been available for comparison. Crude linear fitting of the calculations has suggested that such a zero time extrapolation may be in error by several ohms-square centimeters or more.

DISCUSSION

The behavior of living membranes at short times, such as 10 μs and less, and the instantaneous leakage currents of Hodgkin and Huxley have received relatively little attention (see, however, Goldman and Binstock, 1969; Adelman and Taylor, 1961; Fishman, 1970 *a*) because of both technical difficulties of measurement and a lack of clear guides for their interpretation. As a first step, we have provided a detailed possible description for the apparently passive dielectric membrane transients which are usually the largest component of these short time observations. However, this work may be little more than an early step as the needs for more and better information become more immediate and require more data and more analysis.

It must be emphasized that this entire effort is based upon the empirical expression for the membrane capacity given in Eq. 1. Although no better representation of the experimental results has been found, this is an entirely unreasonable simplification (Cole, 1970) of the more general empirical equation (Cole and Cole, 1941, Eq. 5) which has been widely used, particularly for solid dielectrics. Further, no theoretical basis has been found for the experimental results although it seems possible that long-range interactions may be responsible (Cole, 1965).

Further Work

The present analytical and numerical results need more experimental confirmation before their use can be relied upon. They should be tested over a range of potentials, ion currents, and temperatures, and it may be necessary to consider the dielectric behavior in response to linear and exponential ramp (Fishman, 1969, 1970 *b*) and sinusoid voltage clamps (Palti and Adelman, 1969). In particular the effects of the response characteristics of the current and potential control and compensation systems will have to be calculated as it becomes apparent that they are of significant importance. An obvious omission is the calculation of the effect and the significance of α in determining a membrane capacity (Hodgkin et al., 1952) by the integral $Q_m = C_m V = \int_0^\infty I dt$.

Dr. George H. Weiss of the National Institutes of Health contributed useful suggestions on methods of computation.

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