

ELECTRICAL COUNTING AND SIZING OF MAMMALIAN CELLS IN SUSPENSION

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ABSTRACT A recently developed method of determining the number and size of particles suspended in a conducting solution is to pump the suspension through a small orifice having an immersed electrode on each side to supply electrical current. The current changes due to the passage of particles of resistivity different from that of the solution. Theoretical expressions are developed which relate the current change caused by such particles to their volume and shape. It is found that most biological cells may be treated as dielectric particles whose capacitive effects are negligible. Electrolytic tank measurements on models confirm the theoretical development, and electric field plots of model orifices are used to predict the observed pulse shapes. An equivalent circuit of the orifice-electrode system is analyzed and shows that the current pulse may be made conductivity-independent when observed with a zero input impedance amplifier.

INTRODUCTION

An increasingly popular device (Coulter Electronics, Inc., Hialeah, Florida) counts and sizes suspended particles by using an electric current in a conducting solution confined by a small orifice. The small orifice imbedded in a dielectric sheet connects two volumes of conducting fluid in order both to confine the electrical current flow between the two volumes and to restrict the passage of suspended particles to single events in time. Adding to the usefulness of the resultant device is the fact that the magnitude of the disturbance in the electric current caused by any particle of different resistivity from the conducting solution also bears some relationship to the particle volume and shape. It is the purpose here to develop the theory of the current change in the orifice produced by such particles, to present experimental verification of such considerations, and, finally, in a companion paper, to present experimental results on biologic systems with instrumentation that allows the rapid and accurate measurement of number and size distribution of particles independent of the conductivity of the medium. While mammalian cells have received the greatest emphasis, the over-all aspects of this study are general enough to be applied to any cell or particle whose simple electrical characteristics are known.

THEORY

For many years investigators (1-4) have been concerned both theoretically and experimentally with the electrical properties of cells in suspension. We shall apply the results of such investigations directly to the problem of predicting the current change in the orifice caused by the cell.

Consider a homogeneous spherical cell of radius a and resistivity $\bar{\zeta}_2$ placed in a uniform electric field maintained by electrodes of potential difference V on opposite ends of a cylinder of length $L \gg a$ and area $A \gg a^2$ containing an electrolyte of resistivity ζ_1 . With a cell in the cylinder, the electric field lines form patterns for various cell conductivities as shown in Fig. 1.

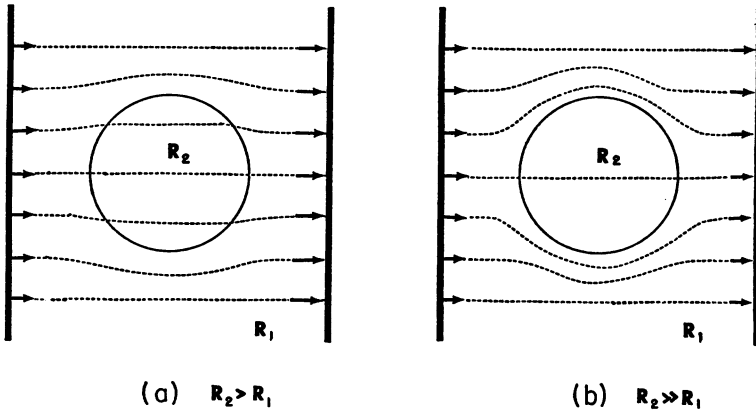


FIGURE 1 Electric field line patterns around spheres of different resistivities.

It can be shown that under these circumstances the apparent resistivity of the fluid in the cylinder becomes

$$\zeta = \zeta_1 \frac{(1 - \beta)\zeta_1 + (\gamma + \beta)\bar{\zeta}_2}{(1 + \gamma\beta)\zeta_1 + \gamma(1 - \beta)\bar{\zeta}_2} \quad (1)$$

where $\beta = 4\pi a^3/3LA$ and γ is a shape factor for the cells. $\gamma = 2$ for spheres and 1 for right circular cylinders parallel to E .

Now, it has been shown that a better electrical model of the cell involves the assumption that the cell membrane is a leaky dielectric of capacitance, C_m , per unit area paralleled by a material of resistivity ζ_m and that the cytoplasm is a reactance-free electrolyte of resistivity ζ_2 . Under these circumstances the resistivity of the cell $\bar{\zeta}_2$ becomes complex and may be represented by

$$\bar{\zeta}_2 = \zeta_2 + Z_m/a \quad (2)$$

where

$$1/Z_m = 1/\zeta_m + i\omega C_m.$$

For direct current

$$\bar{\zeta}_2 = \zeta_2 + \zeta_m/a \quad (3)$$

and for high frequencies

$$\bar{\zeta}_2 = \zeta_2.$$

A survey of the electrical properties of cells allows a generalization as to orders of magnitude. The internal resistivity ζ_2 lies in the range of 30 to 3,000 ohm-cm with 300 ohm-cm typical for mammalian cells. The membrane resistivity ζ_m of interphase cells has a range of 1,000 to 10,000 ohm-cm² while the membrane capacitance, C_m , of most cells lies in the range 1 to 2 $\mu\text{f}/\text{cm}^2$. The resistivity of typical media ranges from 20 ohm-cm for sea water, through 100 ohm-cm for mammalian cells, to about 10^4 ohm-cm for fresh water. Considering a typical mammalian cell to have a radius of 10^{-3} cm, $\zeta_2 \approx 300$ ohm-cm and $\zeta_m \approx 10^3$ ohm-cm², we see for the DC case that ζ_m/a is on the order of 10^6 ohm-cm, as is $\bar{\zeta}_2$. Hence, for the practical case, we see that $\bar{\zeta}_2 \approx \zeta_m/a$ and, since $\bar{\zeta}_2 \gg \zeta_1$, equation (1) reduces to

$$\zeta - \zeta_1 = \Delta\zeta = \zeta_1\beta\left(\frac{1 + \gamma}{\gamma}\right) \quad (4)$$

for $\beta \ll 1$.

If we now consider an orifice to be of area A and effective length L , we see that the change in orifice resistance caused by the presence of a cell is

$$\Delta R \approx \zeta_1 L\beta(1 + \gamma)/A\gamma. \quad (5)$$

One of the questions of importance is the influence of the cell membrane capacitance, C_m . In order to assess orders of magnitude, assume the cell to be a right circular cylinder of radius a and length a parallel to E . Since the field lines inside the cell are parallel to its length, we see that the cell may be approximated by a capacitance of $\pi a^2 C_m/2$ in series with a resistance of $\zeta_2/a\pi$, assuming no membrane leakage. This produces an effective time constant of $a\zeta_2 C_m/2$ for the cell alone.

If we now consider such a cell immersed in a fluid of conductivity ζ_1 , to be suddenly placed in a region where a longitudinal gradient of the electric field exists, it follows that the above time constant must be modified by the resistivity of the surrounding electrolyte. To a first approximation, we may assume that fluid connecting the ends of the cell is equivalent to another restriction about equal to that of the cell itself before field divergence renders it negligible. Then, $T \approx aC_m(\zeta_1 + \zeta_2)/2$. For typical mammalian cells we find $T \approx 4 \times 10^{-7}$ seconds. Cole (1) has shown that a more general expression for cells of any shape is

$$T = \left[\frac{1 + \gamma\beta}{\gamma(1 - \beta)} \cdot \zeta_1 + \zeta_2 \right] C_m a$$

which produces time constants on the same order of magnitude. Since for the apertures and fluid flows used, pulse lengths of 15 μsec . with 5 μsec . rise times due to the

field gradients were observed, it can be concluded that the effect of membrane capacitance for the cells under these circumstances may be neglected. This will be supported by experimental data later. It must be emphasized, however, that this may not be true for all cells and/or particles and care must be exercised when counting a variety of cells.

ORIFICE CHARACTERISTICS

As mentioned, the orifice serves a dual purpose; to confine the electric current and to restrict the passage of particles to single events in time. While β must be less than unity, the orifice cannot be too large relative to the size of the particle since the resultant signal due to the passage of one particle may approach the electrical noise in the system. On the other hand, the orifice cannot be too small in diameter since this both restricts fluid flow requiring higher pressure differentials for a given flow rate and leads to plugging by debris in the liquids. Similarly, the orifice cannot be too thin in the direction of flow since there would be no appreciable distance along the direction of flow in the orifice where the electric field is a constant—a necessary requirement for flat-topped electrical pulses whose height should bear a known relationship to the volume.

The presence of the orifice will modify the equations developed previously—particularly regarding dependence of output voltage change on volume and shape of cells since the cells are no longer small with respect to the boundaries. Consider a spherical non-conducting cell of radius r_1 , on the axis of a tube of radius r_2 , containing conducting fluid of resistivity ζ_1 , as shown in Fig. 2. If we neglect the slope of the electric field at the surface of the sphere, we see that the resistance of each disk dx in thickness and $\pi(r_2^2 - r^2)$ in area is

$$dR = \zeta_1 dx / \pi(r_2^2 - r^2)$$

which leads to

$$\frac{\pi R}{\zeta_1} = \int_{-r_1}^{r_1} \frac{dx}{r_2^2 - r_1^2 + x^2}$$

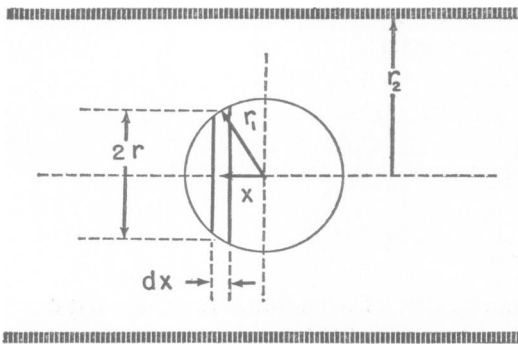


FIGURE 2 A spherical non-conducting cell of radius r_1 on the axis of a tube of radius r_2 .

This is the method of lower limits as used by Smyth (5) and represents the smallest value of resistance to be expected. Evaluating the above integral and taking the difference between the sphere in and out of the tube, we have

$$\frac{\pi \Delta R}{\zeta_1} = \frac{2}{r_2} \left[\frac{\tan^{-1} [K(1 - K^2)^{-1/2}]}{(1 - K^2)^{1/2}} - K \right] \quad (6)$$

where $K = r_1/r_2$. Expanding the term in brackets and defining δ as the volume of the sphere, then

$$\Delta R \cong \frac{\delta \zeta_1}{\pi^2 r_2^4} \left(\frac{1 + 0.3 K^2 + 0.13 K^4 + \dots}{\sqrt{1 - K^2}} \right) \quad (7)$$

From which, for $K \ll 1$,

$$\Delta R \cong \zeta_1 L \beta / A \quad (8)$$

where L , A , and β are as defined previously. Except for the factor $(\gamma + 1)/\gamma = 3/2$, this is identical with equation (5). Since equation (6) is a lower bound, we would expect some difference. The question remaining is the validity of the dependence on K . Fig. 3 is a plot of equation (6) multiplied by $3/2$ compared with experimental

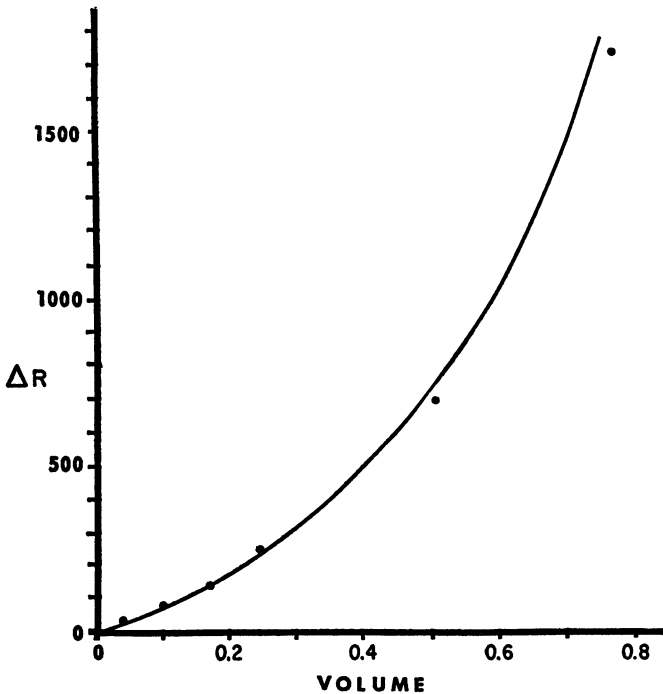


FIGURE 3 The change in resistance in arbitrary units *versus* the volume of spheres (normalized so that at 1.0 the diameter of the sphere equals the diameter of the orifice). The solid line is the theoretical equation (6), and the points are model measurements.

points from model measurements to be explained later. The agreement is quite reasonable. Also, as will be shown later, long thin rods drawn parallel to their long axis will not involve approximations for the electric field at the boundaries and data (Fig. 5) comparing such rods with spheres of the same volume shows that expression (6) for spheres must be multiplied by 3/2 to agree on an absolute basis. Thus, we shall assume that the proper expression for spheres is equation (6) or (7) multiplied by 3/2. Data on absolute calibrations will be presented in a companion paper.

The above equations and assumptions are expected to hold for spheroids but will break down for short rods and disks since current flow lines will not follow flat surfaces when they are normal to the flow of current (*i.e.*, "end" effects will predominate for short disks and rods). Consider first a rod (or disk) of length t and radius r_1 coaxial with an orifice of radius r_2 . By the method used previously, one can show that

$$\Delta R = \frac{\zeta_1 t K^2}{\pi r_2^2 (1 - K^2)} = \frac{\zeta_1 \delta}{\pi^2 r_2^4} \cdot \left(\frac{1}{1 - K^2} \right) \quad (9)$$

where δ is the volume of the rod. While this expression is correct for very long rods ($t \gg r_1$), it does not hold for $t \approx r_1$ because of the end face effect. Experimentally, one finds that a very thin disk moved parallel to its axis produces a signal approximately three times that of an equivalent volume sphere. This means that it is producing a signal 9/2 larger than the above equation predicts. If we ascribe this to an effective insulating cylindrical volume in front of each face equal in area to the area of the face and proportional in depth to the radius of the face, we may modify equation (9) to be

$$\Delta R = \frac{\zeta_1}{\pi^2 r_2^4} \left[t\pi r_1^2 + \sigma\pi r_1^3 \right] \cdot \left[\frac{1}{1 - K^2} \right]. \quad (10)$$

For the same disk traveling through the aperture in a direction parallel to its two faces, it can be shown that

$$\Delta R = \zeta_1 \delta / \pi^2 r_2^4. \quad (11)$$

Again, however, the sharp edges modify this and experimentally we find the disk under these circumstances to produce the same resistance change as a sphere of the same volume. This means that equation (11) must be multiplied by 3/2 to agree with the experiment. Generally, the geometric shapes encountered and the ensuing mathematical complexity of any theory are so limiting that the only recourse is to model experiments for any given set of conditions.

In order to ascertain the field gradients as well as to evaluate both the expected pulse size and shape with the size and shape of the particles, an electrolytic tank model of the orifice was constructed with a scale factor of about 500. Since the electric field is symmetric about the center line of the orifice, only one-half of the orifice

was used, allowing easy access from the top surface for the potential probe. Typical equipotential plots for two different orifices are shown in Fig. 4 where the edge gradients may be seen to be rather small.

By passing a constant direct current through the model orifice and recording the potential change induced by a wax model moving through the orifice, it became possible to demonstrate the dependence of voltage change on the volume of the model, the influence of the shape of the model, and, finally, the expected pulse height with position (*i.e.*, pulse shape, provided the velocity is constant). Since the fluid motion induced artifacts in the output signal and no surface probes were needed, the whole cylindrical orifice was immersed between the two volumes of electrolyte and the wax models slowly pulled through.

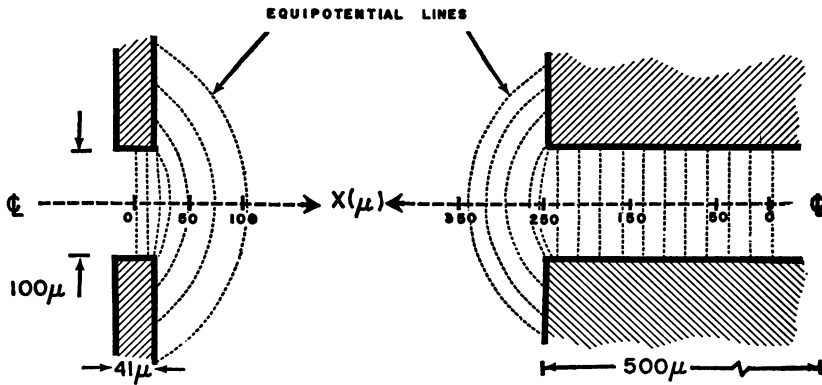


FIGURE 4 Typical equipotential plots for two different model orifices.

Fig. 3 shows the agreement between the resistance change for various spheres and equation (6). Fig. 5 shows the resistance change for two right circular cylinders of constant volume whose aspect ratios change over a range of 30:1 producing an orifice resistance change of only 3:1. The single points shown are for spheres of the same volume as the two cylinders. The ratio of the change for very small diameter rods to that for an equivalent sphere is 0.63 in both cases while the ratio of equation (9) to the modified equation (7) is 0.67 for $K \ll 1$. If we define the aspect ratio to be $\alpha = 2r_1/t$ and substitute this in equation (10) we find

$$\Delta R = \frac{\zeta_1 \delta}{\pi^2 r_2^4} \left(1 + \frac{\alpha \sigma}{2} \right) \left[\frac{r_2^2}{r_2^2 - \left(\frac{\alpha \delta}{2\pi} \right)^{2/3}} \right]. \quad (12)$$

For the experiment shown in Fig. 5

$$\left(\frac{\alpha \delta}{2\pi} \right)^{2/3} \ll r_2^2,$$

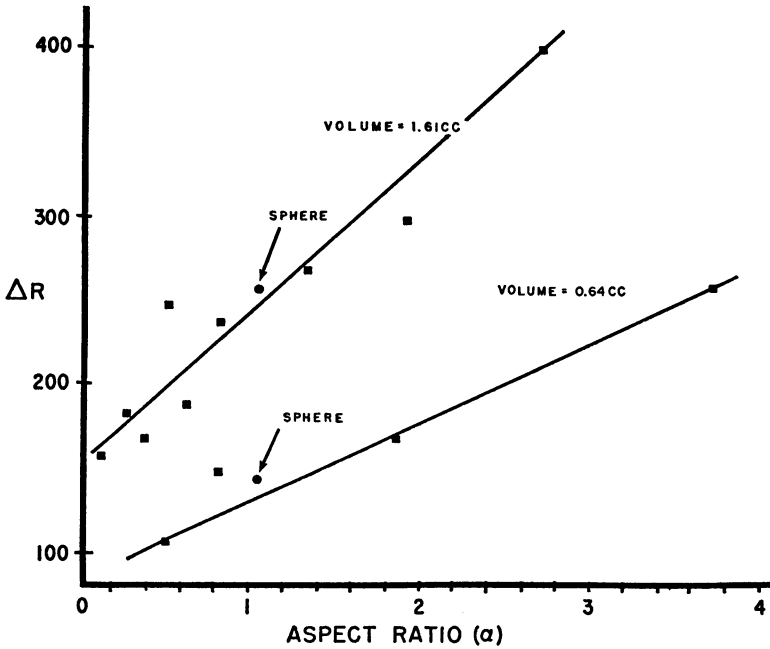


FIGURE 5 The change in resistance in arbitrary units *versus* aspect ratios of cylinders of constant volume pulled through along their axis of circular symmetry.

so that we may neglect the last term and obtain

$$\Delta R = \frac{\xi_1 \delta}{\pi^2 r_2^4} \left(1 + \frac{\alpha \sigma}{2} \right). \quad (13)$$

This shows a linear relation between resistance change and α as observed experimentally; evaluation of the data produces $\sigma = 1.1$ in both cases. To further prove this point, 2.5 cm diameter disks were pulled through a 5 cm orifice along their axis of circular symmetry and the relative voltage change was observed as a function of thickness. The data is plotted in Fig. 6 where the straight line predicted by equation (10) is observed. Evaluation of this data produces $\sigma = 1.16$. Fig. 7 shows the relative resistance change for a disk moved through an orifice both on edge and along its axis of circular symmetry. For the latter case, the units are $\delta/(1 - K^2)$ while in the former case, the units are δ . Both cases produce straight lines as predicted by equation (11) multiplied by 3/2 and equation (12) for a constant aspect ratio of 4. The measured slope ratio is 2.0 as compared with a calculated value of 2.1. These data for disks are of value when considering the dynamics of counting red blood cells. However, evidence indicates that the 3/2 factor is not necessary for human erythrocytes because of their rounded edges.

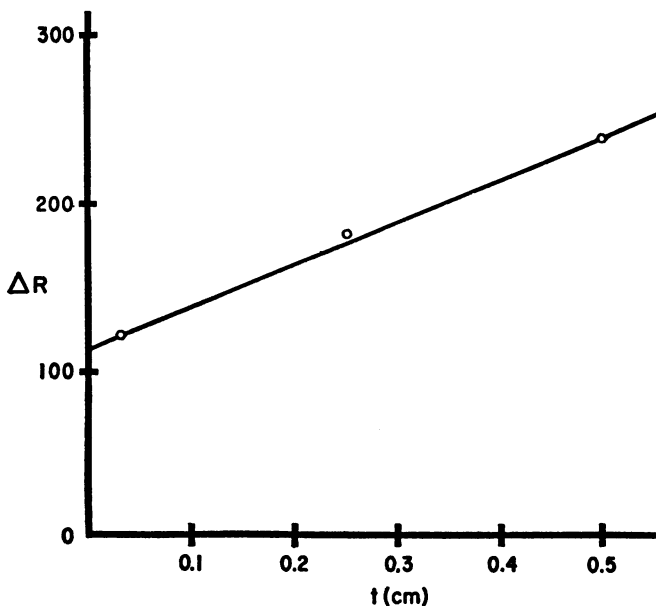


FIGURE 6 The change in resistance in arbitrary units *versus* the thickness of disks pulled through the model orifice along their axis of circular symmetry.

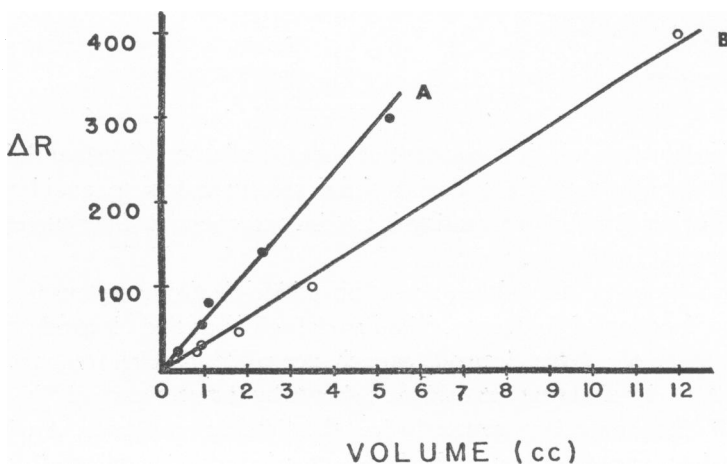


FIGURE 7 The change in resistance in arbitrary units *versus* (a) the volume/ $1-K^2$ for disks pulled through along their axis of circular symmetry and (b) the volume for disks pulled through the model orifice on edge.

ELECTRODES, COUNTING SOLUTIONS, AND INPUT CIRCUITS

The electrodes in a typical counter are platinum with a geometry as indicated in Fig. 8. The counting solution is generally normal saline solution; however, in many cases, the tissue culture medium itself is used. When one suddenly applies a constant current to the electrode system, there is a rapid potential rise followed by a relatively slow increase in potential that approaches a constant after several minutes of operation. The height of the final plateau is also a function of the electrode current. Removal of the electrode current shows the presence of residual potential that dimin-

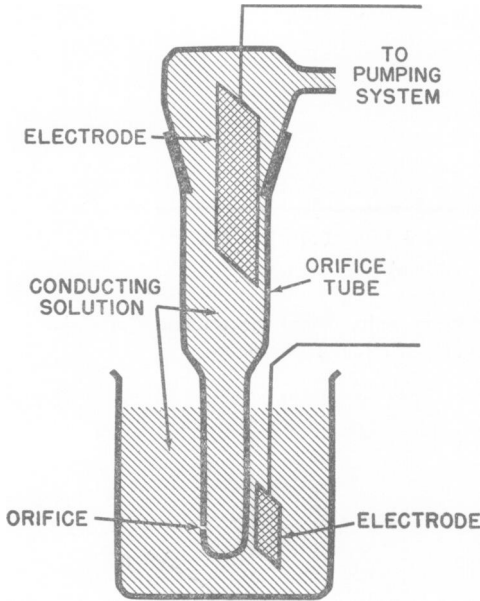


FIGURE 8 Typical geometry of the orifice-electrode system.

ishes with an open circuit time constant of about 6 minutes. Constant fluid flow was maintained through the orifice during these measurements to avoid heating the fluid in the orifice itself. Such heating is not inconsequential since current densities on the order of 1 amp/cm^2 are used.

Fig. 9 is a graph of the voltage across an orifice-electrode system for a constant current as a function of frequency. The first fall off is due to electrode polarization and the presence of a large electrode capacitance while the high frequency fall off is due to the capacity shunting the orifice as shown by the geometry of Fig. 8. Because of its small size, the orifice generally has the limiting resistance. Analysis of the impedance data for a 95μ diameter by 70μ long orifice filled with liquid, then blocked by an insulator, and finally blocked by a conductor produces the equivalent circuit shown in Fig. 10 (a) where $R_0 = 12,000$ ohms, $R_i = 3,000$ ohms, $R_e = 2,000$ ohms, $C_e = 130 \mu f$, $C_o = 83 \mu \mu f$, and $E_e \approx 2.0$ volts for a solution of 70 ohm-cm resistivity.

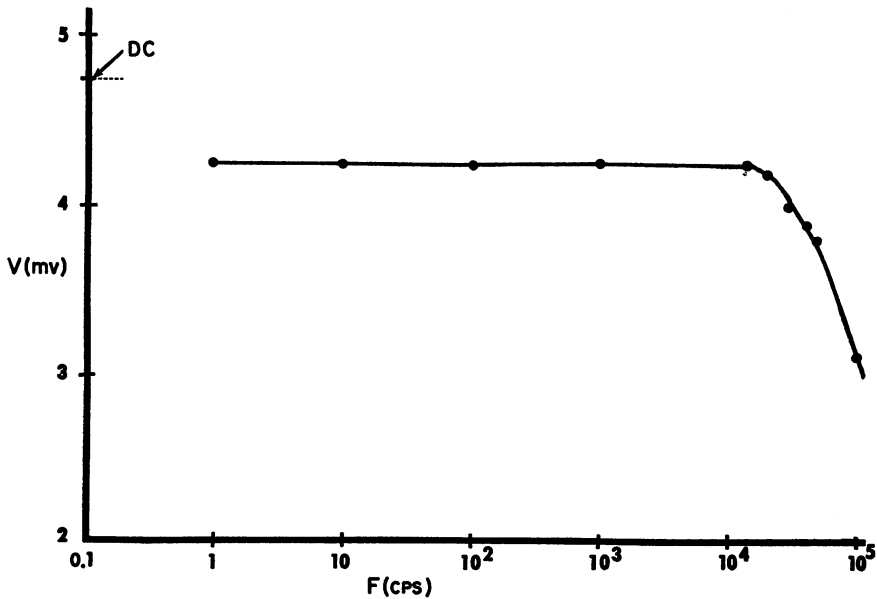


FIGURE 9 Voltage *versus* frequency for the orifice-electrode system of Fig. 8. The calculated DC voltage value shown is for the equivalent electrical circuit of Fig. 10 (a) with E_e removed.

R_l is the resistance of the liquid columns connecting the orifice to both electrodes and R_e is the polarization resistance at the electrodes. For the pulses produced by a cell traversing our present orifice (rise times of about $5 \mu\text{sec.}$ and plateaus up to $15 \mu\text{sec.}$ long), we see that we may neglect all capacitances and batteries and be concerned only with the circuit shown in Fig. 10 (b) which additionally shows the input circuit of an amplifier ($C_{in} + R_{in}$) and a source of orifice current (constant current from an infinite impedance source). If the amplifier has a large input impedance, then

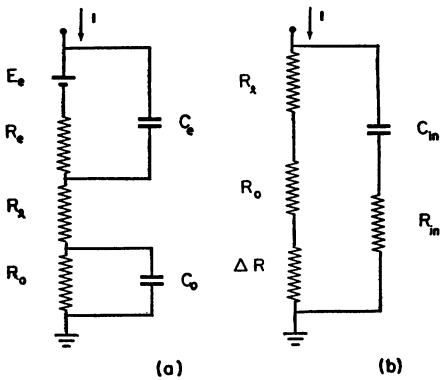


FIGURE 10 Equivalent electrical circuit of orifice-electrode system. (a) R_e , resistance of orifice; R_l , resistance of liquid; R_e , resistance of electrodes; C_e , capacitance of electrodes; C_o , capacitance of orifice; I is a constant current; and E_e , the electrode battery. (b) $C_{in} + R_{in}$ is the input circuit of the amplifier.

the change in voltage appearing across the input is $\Delta V = I_0 \Delta R$ and since the fluid conductivity is involved in ΔR , the signal is conductivity dependent.

Consider now the case where C_{in} is very large and R_{in} very low followed by a current amplifier (*i.e.*, a "zero" input impedance amplifier). C_{in} obviously blocks the steady-state orifice voltage but allows any change in current to pass into the amplifier. Similarly, C_{in} will maintain the voltage constant during the passage of any particle in spite of the slower "constant" current source. From Ohm's law, for constant voltage,

$$dI = -V dR/R^2 = -I dR/R$$

so that $\Delta I/I = \Delta R/R$

where R is the total resistance (orifice plus fluid columns) in the circuit and I is the current just prior to the pulse. Since both R and ΔR involve the fluid conductivity, it follows that ΔI is independent of conductivity. For example, for very small spheres ($K \ll 1$)

$$\Delta I = 3I\delta/2G\pi^2r_s^4 \quad (14)$$

where $R = G\zeta_1$.

For larger spheres one must use equation (6). Using a zero input impedance amplifier and the electrolytic model discussed previously, a fractional current change of 0.0020 was observed for a wax sphere of 0.75 cm diameter moved through an orifice 5 cm in diameter by 7 cm long. As would be expected, this ratio was found to be independent of I and the solution conductivity over extremely large ranges. Equation (14) produced $\Delta I/I = 0.0024$ for $G = L/A$ which means that assuming the orifice to be the controlling resistance yields an error of 20 per cent in $\Delta I/I$ in this case. The experimental value of G (measured at 1000 CPS) produced the expected value of ΔI within 5 per cent.

To demonstrate that cell membrane capacities are negligible, mouse lymphoblast cells (L-5178Y), latex particles (Bioproducts Department, The Dow Chemical Company, Midland, Michigan) and pollen spores were pumped through two apertures of different lengths (95 μ diameter by 70 μ long; 80 μ diameter by 500 μ long) and the resultant wave shapes observed with a 0.1 μ sec. rise time amplifier. While a number of odd shapes of the correct peak voltage were observed with the shorter aperture (due probably to non-axial paths), the majority had flat tops and rise and fall times directly attributable to the axial electric field distribution shown previously. The pulses for the longer aperture also were as expected for an axial dielectric particle with rise and fall times due to the edge gradients. Fig. 11 compares observed wave shapes with those predicted mathematically from the field distribution and those observed from the electrolytic tank model. The close check of all three approaches demonstrates reasonably well that cell membrane capacities may be neglected under these circumstances.

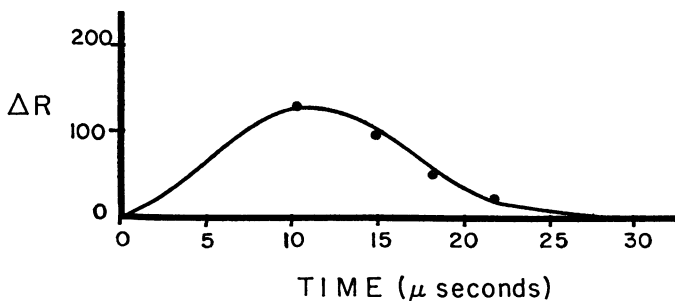


FIGURE 11 Observed pulse height *versus* time for latex particles and mouse lymphoblast cells pumped through a 95μ by 70μ orifice. Latex particles and mouse lymphoblast cells show the same pulse shape to about 5 per cent (solid line). Drawings are from oscilloscope films. The points are the theoretical values calculated from the field distribution and based on optically measured diameters of mouse lymphoblast cells. The electrolytic tank pulse fits the latex pulse too closely to be differentiable on this figure.

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REFERENCES

1. COLE, K. S., and CURTIS, H. J., Electrical physiology: electrical resistance and impedance of cells and tissues, *in* Medical Physics (O. Glasser, editor), Chicago, Year Book Publishers, Inc., 1944, 1, 344.
2. COLE, K. S., and CURTIS, H. J., Bioelectricity: electric physiology, *in* Medical Physics (O. Glasser, editor), Chicago, Year Book Publishers, Inc., 1950, 2, 82.
3. SCHWAN, H. P., and COLE, K. S., Bioelectricity: alternating current admittance of cells and tissues, *in* Medical Physics (O. Glasser, editor), Chicago, Year Book Publishers, Inc., 1960, 3, 52.
4. COLE, K. S., *J. Gen. Physiol.*, 1938, 15, 64.
5. SMYTH, W. R., Static and Dynamic Electricity, New York, McGraw Hill Book Co., 1950, 2nd edition.