Aspects of Cell Size Measurement in *Tetrahymena elliotti*

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*Tetrahymena elliotti* was used to assess the accuracy of the volume distribution measured with a Coulter counter. The volumes of the cells were shown to be underestimated because of their electrical conductivity. Theoretical considerations predicted the observed excessive skewness of the distributions. It is concluded that the only reliable parameter obtained from a Coulter volume distribution is the mean size.

**INTRODUCTION**

The measurement of cell size and cell numbers has gained a great deal in popularity in recent years with the advent of electronic methods of measurement, such as the Coulter counter and Channelyzer, the Celloscope, etc. These techniques are most frequently used to obtain an estimate of biomass, but occasionally the volume distribution has also been studied. The aim of this paper is to evaluate the use of a Coulter counter to obtain volume distribution data and to indicate and discuss the underlying assumptions which are made. While the employment of electronic devices of this nature to count numbers of particles presents few problems (Rasmussen et al., 1974), the evaluation of particle volumes is not so straightforward. A Coulter counter operates by drawing a suspension of particles through a constriction between two electrodes (Helleman, 1972). Simplistically, the size of the resistance change (pulse height) observed when a particle enters the orifice was said to be proportional to the volume of the particle (Harvey & Marr, 1966; Kubitschek, 1960). However, a detailed study (Ben Sasson et al., 1974; Grover et al., 1969a, b, 1972) has shown that the shape of the particle (and its orientation if it is non-spherical), its resistivity and the current density in the space it occupies also affect the pulse height. It is conceivable that the exact consequences of these variations could be calculated but the fate of the pulses in the electronic circuitry of a Coulter counter and Channelyzer is not sufficiently well understood to allow a prediction of the final shape of the volume distribution. The accumulation of volume distribution data without the Channelyzer is of limited usefulness because of counting errors, the time involved, the size of sample required and the two types of coincidence which may occur (Curds et al., 1978). The only course left, therefore, is to compare the distributions obtained using the Coulter counter and Channelyzer with other techniques which may yield an acceptable standard.

**METHODS**

The cells used in this study were *Tetrahymena elliotti* Nanney & McCoy, 1976 (previously known as *T. pyriformis* GL, Phenoset B, Borden et al., 1973) obtained from the Culture Centre of Algae and Protozoa, Cambridge (code L1630/1c). Stock cultures were maintained in medium containing proteose peptone (Oxoid; 10 g l⁻¹) and yeast extract (Oxoid; 2.5 g l⁻¹) at 18 °C. Experimental cultures were grown in medium containing proteose peptone (0.5 g l⁻¹), yeast extract (0.125 g l⁻¹) and glucose (0.2 g l⁻¹) at 25 °C in orbital shake flasks (25 to 100 ml; culture volume was one-tenth that of the flask). The growth yield of experimental cultures varied linearly with the glucose concentration up to at least 0.6 g glucose l⁻¹. Cells
were generally sampled 20 to 30 h after inoculation, which was between the late-exponential phase and early-stationary phase under these conditions. Cell numbers were determined with a Coulter counter and were about $2 \times 10^4$ ml$^{-1}$.

Electronic measurements of cell numbers and size were made with a Coulter counter (model Fn), a Channelyzer, (model CI000) and a teletype interface (Coulter Electronics, Coldharbour Lane, Harpenden AL5 4UN). One vol. culture was diluted with 9 vol. saline to a final concentration of 0.5 g NaCl 1$^{-1}$ (8.6 mm). All saline solutions were membrane filtered (0.1 pm pore size) on the day they were used. Counts were corrected for coincidence using the expression $N_e = N_o e^{-\psi \lambda}$, where $N_o$ is the observed count, $N_e$ is the true count and $\psi$ is the coincidence correction constant (Curds et al., 1978). Background counts were less than 10 and were considered insignificant compared with cell counts which were of the order of 2000 in 0.5 ml. Volume distributions were accumulated until the distribution form was smooth and contained at least 100 cells in the mode channel. A standard orifice of 150 pm nominal thickness, and a special ‘high resolution’ tube (Mundschenk et al., 1976), with a nominal thickness of 210 pm, were used; both were 200 pm in diameter. The apparatus used for hydrodynamic focusing (Adams & Gregg, 1972) was constructed according to the description of Mundschenk et al. (1976) and von Behrens & Edmondson (1976). Calibration particles (pollen) were supplied by Coulter Electronics.

Cells for optical measurement were prepared by placing a drop of culture within a ring of petroleum jelly on a plain microscope slide which was then inverted for 10 s over 2% (w/v) aqueous osmium tetroxide. A coverslip was placed over the drop, supported by the petroleum jelly; this prevented both evaporation and deformation (by crushing) of the protozoa. Immediate measurements were made using a Vickers Image Splitting Eyepiece (Vickers, Purley Way, Croydon CR9 4HN) modified according to Rifkin (1968) and linked to a teletype via a foot switch. Measurements of up to 350 cells could be completed within 1 h, at an accuracy of ± 2 pm, during which time no significant change in overall cell dimensions could be detected.

**RESULTS**

**Cell shrinkage during measurement**

It has been widely reported that the nature of the diluent affects the volumes of erythrocytes measured with a Coulter counter (for review, see Helleman, 1972). *Tetrahymena elliotti* cells were measured live, in NaCl solutions, since fixation led to unreproducible values for the mean cell volume (MCV), even under rigidly controlled conditions (C. H. Wu, personal communication). The MCV recorded on the Coulter system was found to be dependent on the concentration of NaCl used as diluent (Fig. 1). Cells began to shrink very rapidly after dilution (Fig. 2), then maintained a steady volume for about 40 min. The initial rate of shrinkage was constant for the range of salinities tested, but the plateau region was lower for higher concentrations of NaCl. The lowest concentration of NaCl usable in our Coulter counter was 0.5 g l$^{-1}$ (8.6 mm), and this was used for all further measurements. Other salts (KCl, MgSO$_4$, sodium acetate) at the same molarity gave results indistinguishable from those in Fig. 2. Incorporation of any of these salts in the growth medium caused a severe lowering of growth rate and total yield. Cells grown in media with added mannitol (8.6 mm), which was not utilized by *T. elliotti* since the growth rate and total yield were unaffected by its presence, also gave results indistinguishable from those in Fig. 2. The addition of albumen (egg or bovine) at 0.3 g l$^{-1}$ (Schmid, 1967) had no significant effect on the size change observed and greatly increased the background counts because of coagulation after filtration.

**Cell shape considerations**

The shape factor is a function of length and breadth for ellipsoids and, using the expressions of Grover et al. (1969a) or Hurley (1970), varied between 1.20 and 1.50 with a mean of 1.33 for a single culture of *T. elliotti* cells (350 observations). The mean values for 12 cultures varied between 1.26 and 1.34, with a mean of 1.30. Variations in the shape factor within a population will have a small effect for aligned cells (those whose major axis is parallel to the orifice axis) but if the cells tumble as they pass through the orifice region then the effect can be rather more severe (Grover et al., 1972). The variation in the shape factor will add to the width of the distribution, increasing the standard deviation and the skewness.
Cell size in *Tetrahymena*

**Fig. 1**. Mean cell volume of *Tetrahymena elliotti* measured with the Coulter counter using various concentrations of NaCl as a diluent. The Coulter size was calculated by calibration from Fig. 3. A delay of 5 min was always allowed between dilution and measurement.

**Fig. 2**. Shrinkage of *Tetrahymena elliotti* after dilution with 8.6 mM-NaCl. The ordinate is the percentage of cells larger than 16900 μm³ (calibrated from Fig. 3), which will be related to the mean cell volume in a linear fashion if the distribution form is not changing.

**Optical measurement**

If the length \((L)\) and breadth \((B)\) of a cell are measured the volume may be calculated on the assumption that the shape of *T. elliotti* is a prolate spheroid: the volume \((V)\) is given by

\[
V = \frac{4}{3} \pi \cdot \frac{L}{2} \cdot \left(\frac{B}{2}\right)^3 = \frac{\pi}{6} \cdot L \cdot B^3
\]

An alternative method of calculation (Taylor & Berger, 1976) uses the projected cell area. The projected cell area of *T. elliotti* was highly correlated to the product of length and breadth (correlation coefficient, \(r^2 = 0.968\)), and there was no significant difference between the volumes calculated by either method.

Measurements of cell dimensions from silver-stained specimens prepared by the Chatton-Lwoff method (Corliss, 1953) showed no significant difference in mean cell length, but the mean cell breadth was significantly reduced compared with freshly fixed cells from the same population.

**Comparison of electronic and optical mean cell volume measurement**

The MCV measured by the Coulter system was linearly correlated \((r^2 = 0.72)\) to the mean cell volume calculated from optical measurements (Fig. 3). Calibration of the Coulter counter with pollen of known size gave MCV values 4-fold lower than those measured optically.

**Comparison of electronic and optical volume distribution measurement**

The volume distribution of a population of *T. elliotti* measured optically and with the Coulter system showed a fundamental difference in form (Fig. 4). The electronically derived distribution was considerably broader at the base, possessing a significantly longer tail. This tail was probably generated by cells passing through regions of high current density.
Fig. 3. Correlation of mean cell volumes measured with the Coulter counter with volumes calculated from microscopical measurements of at least 100 cells. Cell size variation was achieved by sampling several batch cultures at different times. The line of best fit was calculated from bivariate regression giving: Optical size = 539.7 \times \text{Coulter size} + 5436; \text{correlation coefficient, } r^2 = 0.72.

Fig. 4. Comparison of the form of the volume distribution measured by microscopy (histogram) with that measured by the Coulter counter (○). The scale of the Coulter distribution has been adjusted to give the best visual fit.

Fig. 5. Volume distributions of *Tetrahymena elliotii* obtained using a Coulter counter fitted with a hydrodynamic focusing system (●) and without such a system (○). Both distributions are plotted on the same scale.

Fig. 6. Volume distributions of *Tetrahymena elliotii* obtained using a normal (150 \(\mu\)m thick) probe (●) and a ‘high resolution’ (210 \(\mu\)m thick) probe (□). Both distributions are plotted on the same scale.

(Grover et al., 1969a) appearing substantially larger than if they had passed through the axial region (Thom et al., 1969; Kachel, 1976). This effect, together with the effect of tumbling, causes an overestimate of the cell size, weighting the distribution to the right-hand side and increasing the apparent skewness.

Adams & Gregg (1972) used a system of hydrodynamic focusing to constrain the cells to a
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particular path through the orifice zone. The use of an adaptation to a standard Coulter counter to permit hydrodynamic focusing (see Methods) resulted in considerably smaller cell volume distribution curves (Fig. 5). When the scale was adjusted to compare the focused distribution with the optically derived data (see Fig. 7) the distributions were seen to have similar forms.

The use of 'high resolution' orifice tubes (Mundschenk et al., 1976) was introduced to prolong the residence of the particle being sized in the sensing zone (Grover et al., 1972), and hence increase the duration of the pulses generated. This improves the signal-to-noise ratio and makes the measurement of the pulse height more accurate. For T. elliotti the effect of an increase in the length of a 200 μm diameter orifice from 150 μm to 210 μm was to make the distribution slightly narrower at the base (Fig. 6). The effect of this change was expected to be small since Grover et al. (1972) recommend the length of the orifice to be twice its diameter (i.e. 400 μm in this case) and the prime source of error is the inhomogeneity of the electrical field near the edges of the orifice.

DISCUSSION

The phenomenon of cell shrinkage after dilution has been studied by Morrison & Tomkins (1973), who photographically measured a single cell of T. pyriformis (strain W) and found that its volume changed in the same manner as the MCV measured with the Coulter counter. The diluent was used at 12 g l⁻¹ (0.205 M), which was osmotically much stronger than the diluent used here. Rifkin (1973) showed that T. pyriformis (strain W) did not change its MCV due to osmotic pressure in buffer solutions but adjusted the output of its contractile vacuole in response to changing external osmotic pressure. Since, in the present work, the addition of mannitol (equimolar with the diluent) to the growth medium did not change the shrinkage curve, it is unlikely that the observed shrinkage is attributable to osmotic effects.

Marine protozoa lacking a contractile vacuole use a sodium pump to expel excess water (Kitching, 1967). Since the same shrinkage response was observed in the present work using Mg²⁺ and K⁺ as well as Na⁺, it is unlikely that sodium pump stimulation is responsible for the apparent loss of volume.

The most plausible explanation for the shrinkage observed is that the cells were becoming more conductive. Dunham & Child (1961) reported that Tetrahymena is highly permeable to metal ions, and Fricke (1924, 1953a, b) discussed the effect of increasing conductivity of particles in suspension on the resistance of the suspension. The theory of size measurements using the Coulter principle normally assumes cells to be of high resistivity compared with their environment, and they are usually treated as insulators. Since water expulsion is not considered to be a likely reason for the cell shrinkage observed, it is reasonable to question the assumption that the cells are of low conductivity. An increase in the conductivity of the cell will result in a lower resistance pulse observed as it passes through the orifice zone of a Coulter counter, and the greater the conductivity of the cell, the lower the pulse observed. Dilution of T. elliotti cultures with increasing concentrations of NaCl showed decreasing observed MCV values (Fig. 1). The rate of shrinkage was indistinguishable for all concentrations of NaCl tested, although the plateau values varied with concentration. It was therefore thought likely that T. elliotti cells were absorbing ions after dilution and thus altering their conductivity.

The high correlation observed between the projected cell area and the product of cell length and breadth in T. elliotti indicated a consistent shape outline. The estimation of the cell volume, however, involves an assumption about the thickness of the cell, i.e. that its thickness is the same as its breadth. Rifkin (1973) observed that if Tetrahymena shrinks rapidly, it does so by longitudinal furrowing of the pellicle, which has a marked effect on the breadth but not on the length. This means that rapid volume changes will be most strongly
Fig. 7. Volume distributions of *Tetrahymena elliotti* measured using the hydrodynamic focusing system (●) and optically (histogram) compared, as described in the text, with a mathematical model (—) derived from an equation of Powell (1964). The model was fitted by maximum likelihood to the Coulter distribution, and both were adjusted to the scale of the histogram.

reflected in the width of the cell, and hence the breadth measurements will have inherently greater variability. Since this measurement is squared in computing the cell volume, a larger error may be expected than if the cell shrank evenly in all dimensions.

The comparison of MCV measured with the Coulter system and with optical methods showed a linear correlation, but it should be noted that the line did not pass through the origin. Calibration with particles of a known size could not allow for this, or for the differences in conductivity and shape between the calibration particles (usually pollens, which are insulators) and the experimental cells. The volume of *T. elliotti* cells measured with the Coulter system calibrated with particles of known volume tended to be less than that measured by optical methods. While this was in part due to the shape factors mentioned above, this alone could not explain the 4-fold differences observed in this laboratory, the 4.9-fold differences observed by Morrison & Tomkins (1973) or the 1.2- to 2.6-fold differences of Ricketts & Rapitt (1974). It should be noted that Morrison & Tomkins (1973) compared methods of measurements in terms of the diameter of a sphere of equal volume to the measured cells and this reduced the apparent error since it is the cube-root of the differences in volumes. These differences could be explained by the greater conductivity of *T. elliotti* compared with the pollen.

Consideration of the theory of unicellular growth and division allows a prediction of the shape of a steady state volume distribution (i.e. when the mean cell size is not changing in a growing population). Collins & Richmond (1962) and Koch & Schaechter (1962) (mathematically corrected by Powell, 1964) both derived expressions for the distribution of cell volumes $[\mathcal{L}(x_0); \text{Powell (1964), equation 16}]$, which may be stated as

$$\mathcal{L}(x_0) = \frac{C}{x_0} \int_{x_0}^{2x_0} l(\xi) \, d\xi$$

where $x_0$ is the cell volume, $C$ is the harmonic mean of $l(x_i)$, and $l(x_i)$ is the volume distribution of the cells about to divide.

If we assume that (i) the individual cells increase in mass in an exponential fashion from one division to the next, (ii) the daughter cells are both exactly half the volume of the parent cell and (iii) the distribution of $l(x_i)$ is normal, with a mean $\mu$ and standard deviation $\sigma$, then the model may be compared to the observed distributions (using a least squares technique to determine $\mu$ and $\sigma$ from the focused Coulter distribution). With the quality of
data available, the model cannot be said to be different from either the optically derived distribution or from the focused Coulter distribution (Fig. 7). The agreement of these three distributions, (the theoretical and two independent measurements) indicates that the observed distributions had the same form as the true distributions of cell volumes.

CONCLUSIONS

The standard Coulter counter, with Channelizer and teletype interface, is a fast, convenient tool for the study of changes in cell volumes, but great care should be exercised in the calibration of the instrument. Ideally, cells (or particles) that are to be sized routinely should be used for calibration and measured by another, independent, method. Selection of the diluent fluid should be made to make the least change in the osmotic and electrolytic environment of the cells, and ideally, if the growth medium is sufficiently conductive, filtered growth medium should be used.

The study of volume distribution properties, other than the mean, is extremely difficult unless a hydrodynamic focusing device is used. The disadvantage of focusing is that cell counts cannot be obtained as there is no way of metering the volume of cell suspension passing through the orifice. Therefore, to measure both the cell numbers and the volume distributions, the focusing attachment must be fitted and removed for each sample, thus sacrificing some of the speed and convenience of the instrument.

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