

CHARACTERIZATION OF SOMATIC CELLS BY DETERMINATION OF THEIR VOLUMES WITH A COULTER COUNTER

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To date the usual method for checking variation among populations of somatic cells grown *in vitro* has been gross microscopic examination. In a previous paper an attempt was made to characterize cell populations in a more quantitative way by measuring cell diameters under the microscope (Simons, 1967). This kind of characterization can be obtained faster and more exactly when an electronic counter is used to determine the cell volumes. Several authors (Peacock, Williams, and Mengoli, 1960; Brecher et al., 1962; Richar and Breakell, 1959) have already dealt with size analysis of mammalian cells. It is suggested here that statistical measures be used routinely to characterize cell populations grown *in vitro*.

As a rule, frequency distributions of cell volumes are skew and not normally distributed. It has often been reported (Peacock et al., 1960; Brecher et al., 1962; Scherbaum and Rash, 1957) that after logarithmic transformation the cumulative frequency distribution plotted on a probability scale is a straight line, indicating a normal distribution. Therefore, the data obtained by size analysis were fed into an IBM computer and after logarithmic transformation the mean, standard deviation, skewness, and kurtosis were computed for the description of cell volume distributions. These parameters express the shape of near normal frequency distributions quite accurately (Mather, 1951). In this paper some technical problems of the application of this method are considered. Cell volumes were determined with a Coulter counter Model B (Coulter Electronics, Hialeah, Fla.), connected to a Coulter automatic cell size distribution plotter. For all cell populations, a 70 μ

orifice tube was used. Care was taken that the number of cells did not exceed 20,000 cells per ml, as in that case coincidence is below 1% for this aperture tube (service manual, Coulter counter) and no coincidence correction need be applied. For the choice of the electrolyte, a balanced salt solution (Tyrode) without calcium and magnesium was thought to be superior to the usual saline in keeping cells in optimal condition during size analysis. Since sometimes cells still appear to clump or to attach to glass in spite of the absence of calcium and magnesium, sodium citrate was added to a final concentration of 0.38% to prevent attachment and clumping. A correction was made for hypertonicity. In the presence of citrate, cells do not attach to the glass; on the contrary, monolayers of cells detach from the glass surface. Henceforth, this salt solution will be referred to as C.S.S. (Counter Salt Solution). The composition of 1 liter of C.S.S. is given in Table I. The solution is filtered through a millipore (0.45 μ) filter and sterilized by autoclaving.

Recently, it was stated (Harvey, 1968) that the Coulter counter as such is not suitable for determination of volume distributions because of the heterogeneity of transit times through the aperture, which distorts the distribution of measured particle volumes. Harvey was working with small latex particles (mean diameter about 2 μ) and an aperture tube of 30 μ in diameter. As Harvey remarked, for larger orifices the heterogeneity in transit times is much less. Therefore, it is not certain whether the same distortion occurs for the volume distributions of somatic cells. This can be checked by measuring a cell suspension with both

TABLE I
The Composition of 1 Liter of Counter Salt
Solution (C. S. S.)

Material	Amount
NaCl	7.83 g
KCl	0.18 g
Na ₂ HPO ₄ ·2H ₂ O	0.045 g
Glucose	1.80 g
Na-citrate	3.80 g
Phenol red	1.5 ml from 1% solution
Water	998.5 ml

The pH is adjusted to 7.0.

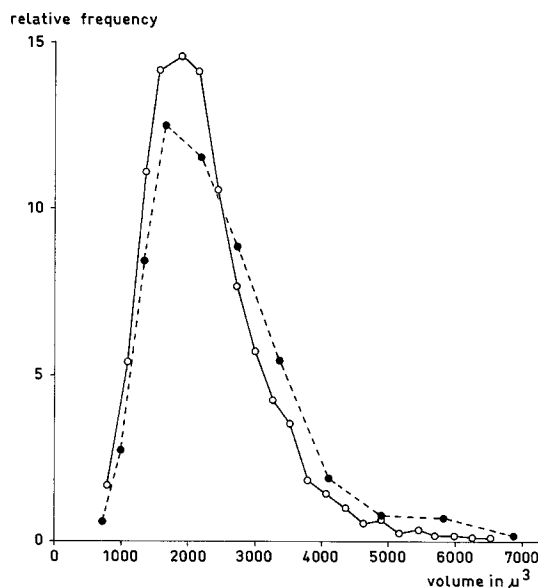


FIGURE 1 Comparison of volume distribution obtained by using the Coulter counter and volume distribution obtained by measuring cell diameters under the microscope. BSC-1 cells. Open circle, Coulter counter data; closed circle, diameter data.

the Coulter counter and the microscope. The shape of the distributions will reveal whether a distortion in the frequency distribution from the Coulter counter occurs. Therefore, a suspension of BSC-1 cells, originally derived from the kidney of the African green monkey (Hopps et al., 1963), was divided into two tubes. One tube was used for measuring 900 cell diameters under the microscope. The cell diameters were measured under conditions described previously (Simons, 1967). Size analysis, with the Coulter counter, was carried out with the other sample. As can be seen in Fig. 1, under these conditions distortion does not occur, which is in agreement with observations by Peacock et al. (1960).

Use of the Coulter automatic size distribution plotter reduces the time required for measurement and eliminates the influence of the operator. To check whether the plotter introduces systematic errors, BSC-1 cells were measured with plotter and without plotter. Three kinds of data were obtained: (1) frequency distributions as recorded by plotter, (2) frequency distributions of the actual counts during measurement with the plotter, (3) frequency distributions of measurements without plotter. Table II shows the parameters from two cell suspensions. Comparison of measurements type 1 with measurements type 2 shows agreement in mean volume, but the standard deviation for measurements of the plotter is lower because some pen deflection exists at low frequencies. Comparison of measurements type 2 with measurements type 3 is indicative of the coincidence of the two linear scales. Here deviations occur in mean volume, which means that the linear scale of the plotter does not coincide exactly with the linear scale of the threshold. By comparison of the cumulative frequency distributions, a regression line between the two scales can be computed. The

TABLE II
Comparison of the Parameters for Two BSC-1 Cell Suspensions

	Frequency distributions	Mean in log μ^3	Standard deviation	Skewness	Kurtosis
Cell suspension 1	Type 1, recording plotter	3.349	0.150	+0.09	-0.28
	Type 2, counts plotter	3.359	0.160	+0.27	+0.04
	Type 3, counts without plotter	3.321	0.161	+0.25	+0.16
Cell suspension 2	Type 1, recording plotter	3.422	0.146	+0.01	-0.11
	Type 2, counts plotter	3.429	0.156	+0.07	-0.19
	Type 3, counts without plotter	3.413	0.158	+0.07	-0.29

TABLE III
Influence of Cell Number per ml on the Estimation of Parameters for Frequency Distributions of BSC-1 Cell Volumes

Parameters	Number of cells per ml C.C.S.				
	16 000	18 000	19 000	20 000	21 000
Mean in $\log\mu^3$	3.200	3.194	3.196	3.196	3.197
Standard deviation	0.142	0.138	0.141	0.142	0.141
Skewness	-0.26	-0.25	-0.25	-0.26	-0.24
Kurtosis	-0.13	-0.11	-0.10	-0.17	-0.09

Each figure is based on 10 measurements.

TABLE IV
95% Confidence-Limits of the Parameters, Obtained from 50 Frequency Distributions of One BSC-1 Suspension

Parameter	95% confidence-interval
Mean	3.1977 \pm 0.0093
Standard deviation	0.1411 \pm 0.0054
Skewness	-0.165 \pm 0.087
Kurtosis	-0.113 \pm 0.141

equation for the regression line is $y = 0.939x + 0.067$. In this formula x indicates the scale of the plotter and y the scale of the threshold dial of the counter. It appears that the two zero points nearly coincide, and that the length of the scale of the plotter is 94% of the length of the threshold scale. After correction of the plotter scale, the parameters obtained from the plotter counts agree with the parameters estimated from the counts without plotter.

Since the standard deviation is somewhat influenced by pen deflection, the number of cells per ml may have an influence on the estimate of the standard deviation. To check this, 50 frequency distributions were made from one suspension of BSC-1 cells. These 50 distributions were divided into five groups, the number of cells per ml being different for each group. The data are presented in Table III. The number of cells per ml may have some influence upon standard deviation, as the standard deviations for the group with 18,000 cells per ml are smaller than the standard deviations for the other groups. Therefore, it is recommended that during an experiment the frequency distributions be based upon the same number of cells. From the same data, 95% confidence-intervals of the parameters were estimated (Table IV). The agreement is very good.

This method of cell size analysis was successfully applied to problems of cell ageing in vitro (Simons, 1970).

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