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Determination of Bacterial Cell Volume with the Coulter Counter

H. E. KUBITSCHEK* AND J. A. FRISKE

Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439-4833

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Two methods were used to determine mean volumes of cells of *Escherichia coli* B/rA in both stationary- and exponential-phase cultures, i.e., (i) electronic measurement with a Coulter Counter-Analyzer system and (ii) biophysical measurement of the total volume and number of cells in sedimented cell pellets. Within experimental errors, the methods gave the same mean cell volumes.

Cell volumes of bacteria in growing cultures are usually determined by one of two very different techniques, i.e., electron microscopic measurement of cell dimensions or electronic measurement with a Coulter Counter-Analyzer system. Cell volumes of *Escherichia coli* B/r have been determined by both methods, and in each case, mean cell volumes were observed to increase exponentially with the growth rate (1; F. S. Trueba, as reported in reference 5). At low growth rates (less than one doubling per hour), both methods led to essentially the same values, but at higher rates (three divisions per hour), cell volumes determined by electron microscopy were almost twice as large as those determined electronically (after correction of the ordinate values for cell volume in Fig. 1 of reference 1, which were inadvertently reported at 10 times the observed values due to a decimal point error). Because this difference between the two techniques could have been due to an artifact of electronic sizing, we examined the performance of the Coulter Counter-Analyzer by comparing cell volumes determined electronically with those determined biophysically from total cell number and volume in sedimented pellets of cells (4).

Cells of several different sizes were obtained from either stationary- or exponential-phase cultures of *E. coli* B/rA grown overnight at 37°C in a shaker water bath in nutrient broth or M9-glucose (10 g/liter) medium. Culture volumes were 160 ml for stationary-phase cultures and 50 ml for exponentially growing cultures. The exponentially growing cultures were refrigerated when their A_{660} reached a value of about 0.3, and the refrigerated cultures were pooled to give total volumes of 700 to 1,400 ml. Cell pellets were collected by centrifugation at 4,000 × *g* for 5 min.

To determine the total volume of cells in each pellet, it was necessary to correct for the interstitial fluid space between the cells. This interstitial space was measured by using ^{14}C-labeled inulin, which is an outer membrane-impermeable solute that does not bind to the cells. Because part of the commercial product adsorbed irreversibly to the cells, labeled inulin ([carboxyl-^{14}C]inulin, 2.7 mCi/g; New England Nuclear Corp.) was purified by preadsorption for 10 min to cells from the same growth medium by mixing 2 ml (1 μCi) with a pellet obtained from 40 ml of cells, centrifuging the culture, and decanting the radioactive supernatant. After this procedure was repeated, the radioactivity of the purified supernatant was determined from 0.05-ml samples added to scintillation fluid (Scintiverse, 4.5 ml; Fisher Scientific Co.) and counted in a scintillation counter (Beckman LS-150; Beckman Instruments, Inc.). To test the efficacy of this procedure, an unlabeled pellet was mixed with purified labeled inulin solution for 10 min. The suspension was centrifuged, the supernatant was decanted, and the newly labeled pellet was suspended in unlabeled, spent growth medium. The centrifugation and suspension in unlabeled medium were then repeated. The radioactivity of the final suspension was less than 0.25% of that in the original suspension. Because the specific activities appear only as ratios in the calculations of amounts of interstitial fluid, the errors in these calculations can be no more than about 0.25%, much less than the observed experimental errors (Table 1).

The interstitial fluid volume in each pellet was determined in three sequential steps. (i) The cells in the pellet were suspended in a known volume (1 to 2 ml) of the purified, labeled inulin solution. The suspension volume was measured to determine the original pellet volume, and duplicate 0.05-ml samples were removed to determine suspension radioactivity and cell number. The suspension was centrifuged again, and the specific activity of the supernatant was determined. This specific activity is identical to the specific activity of the interstitial fluid in the pellet. The total activity of the pellet interstitial fluid was calculated from the differ-

TABLE 1. Average values of pellet interstitial fluid volume, cell volume determined by pelleting, and cell volume determined with the Coulter Counter-Analyzer System

Culture type and medium[a]	Avg pellet vol	Fractional vol ± SE[b]	No. of cells in pellet (10^9)	V_I[c]	V_c[d]
Stationary culture					
M9	0.41	0.31 ± 0.02	6.6	0.43	0.44
	0.47	0.31 ± 0.01	6.2	0.52	0.43
NB	0.10	0.30 ± 0.06	1.9	0.36	0.42
	0.445	0.35 ± 0.08	4.8	0.61	0.65
	0.48	0.30 ± 0.05	4.6	0.73	0.63
Growing culture					
M9	0.475	0.30 ± 0.04	3.9	0.85	0.90
	0.41	0.29 ± 0.03	2.5	1.16	1.22
NB	0.39	0.25 ± 0.05	2.0	1.50	1.65
	0.375	0.32 ± 0.05	1.7	1.53	1.68
	0.54	0.33 ± 0.04	2.0	1.80	1.70
	0.65	0.28 ± 0.02	2.3	2.05	1.79

[a] Minimal M9-glucose medium and nutrient broth (NB) were used for stationary and for growing cultures.
[b] Fractional interstitial fluid volume in pellet from the three different kinds of measurement.
[c] Pellet mean cell volume: average pellet volume × (1 − fractional interstitial fluid volume in pellet)/number of cells in pellet.
[d] Mean cell volume from Counter Counter-Analyzer cell size distribution.

* Corresponding author.

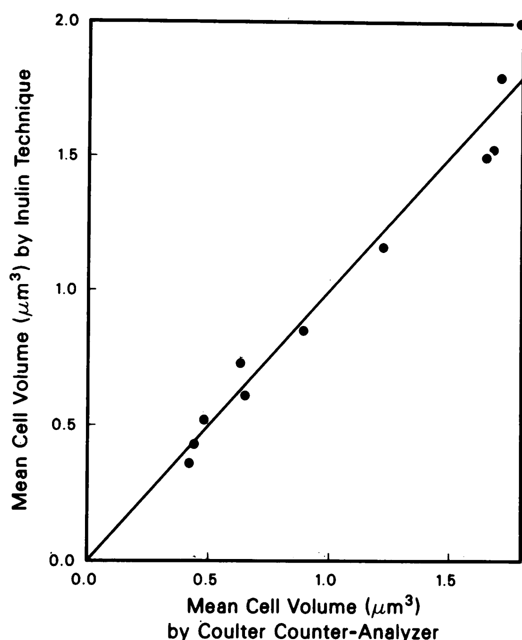

FIG. 1. Mean cell volumes of cultures of *E. coli* B/rA determined by two different techniques. The straight line is the relationship expected when the two methods give the same volumes.

ence between the total activities of the suspension and those of the supernatant, and the interstitial volume was obtained as the ratio of the total radioactivity of the pellet to the specific activity of the interstitial fluid. (ii) The radioactive supernatant was then decanted. The interior surface of the tube above the pellet was carefully cleaned with a cotton swab, unlabeled medium (usually 1.3 ml) was added, and the cells were suspended. The suspension volume was measured, and samples of the suspension were removed to determine specific activity. The total activity of the pellet interstitial fluid was determined from the activity and volume of the suspension. The interstitial volume was calculated as the total pellet activity divided by the specific activity of the supernatant measured in the first step. (iii) The suspension was centrifuged a third time. The activity of the supernatant was measured, and the interstitial fluid volume was determined as in the first step. The average value of the interstitial fluid volume was calculated from the three measurements and subtracted from the total pellet volume to provide the total volume of cells in the pellet. Uncorrected pellet volumes usually were about 0.4 to 0.5 ml, and the average values calculated for the interstitial fluid volumes varied from about 25 to 35% of the pellet volume (Table 1).

Cell numbers were determined with the modified Coulter Counter described previously (2, 3). Samples were sonicated briefly to disperse the cells and suitably diluted for counting in 0.1 N HCl (2, 3). The counter was calibrated for cell numbers by plating appropriately diluted suspensions of viable cells on nutrient agar plates, determining the cell concentrations from colonies counted the following day, and then comparing these numbers with those obtained with the counter. Mean cell volumes were determined by dividing the total cell volume in the pellet by the total number of cells. These values were compared with the mean volumes determined from the cell volume distributions obtained with the Coulter Counter-Analyzer system. The volume scale of this instrument was calibrated with microspheres (1.13 μm diameter) as described previously (3).

Mean cell volumes (V_I) determined by the inulin technique are compared in Fig. 1 to those obtained with the Coulter Counter-analyzer system (V_c). The axes in this figure were chosen with V_c as the independent variable because the precision obtained with the counter-analyzer system was much greater than that obtained with the inulin technique. The straight line in Fig. 1 is the relationship expected when the two methods give the same volumes. When the experimental data in Fig. 1 were fitted by linear regression, the value of the slope (and its standard error) was 1.013 ± 0.073 and the value of the intercept was -0.018 ± 0.086. Thus, neither slope nor intercept differed significantly from the relationship $V_I = V_c$.

In addition, the results in Fig. 1 provide no evidence for a differential sizing alteration with growth rate, as there was no significant deviation between the values from the two kinds of measurements for large, intermediate, or small cells. Nor do the data support the hypothesis that the cell volume measured by the Coulter Counter-Analyzer system is that bounded by the inner membrane of the cell. The periplasmic volume of *E. coli* is about 20 to 40% of the total cell volume (6), so we would have expected average cell volumes to be reduced by this magnitude if volume were delineated by the inner membrane.

In conclusion, the agreement between mean cell volumes measured by the two methods provides evidence that cell volumes determined with the Coulter Counter-Analyzer system are in substantial agreement with the values determined biophysically for the same cells, thereby validating the use of electronic cell sizing for measurements of bacterial volumes. The source of the different response obtained by electron microscopy is unknown, but it would appear that the procedures necessary for electron microscopy might lead to different degrees of cell shrinkage during fixation, depending on cell volume.

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