ELECTRICAL DETERMINATION OF VIABILITY IN SALINE-TREATED MOUSE MYELOMA CELLS

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ABSTRACT Suspension of mouse myeloma cells in phosphate buffered saline (PBS) induced a significant amount of cell death. The lethal effects of PBS include an increase in cell lysis, a decreased ability of cells to exclude trypan blue, and a decrease in the colony-forming ability of these cells. Dead cells were also detected on a Coulter counter by the increase in the fraction of cells with a smaller electrical size distribution (ESD). Comparing mixtures of live and dead cells by ESD and trypan-blue exclusion showed a high correlation of electrical size with viability (correlation coefficient = 0.98). Sizing of PBS-treated cells by light microscopy suggested that the altered ESD of the PBS-treated cells was due to a downward shift in the volume distribution. Light-scattering experiments also suggested a decrease in the size of cells after PBS treatment. An increase in permeability of the cell membrane may also contribute to these results. We conclude that electrical sizing is an excellent indicator of physical changes that result from PBS-induced cell death, and is an effective method for distinguishing live and dead mouse myeloma cells after PBS treatment.

INTRODUCTION

Although dilute suspension of cells in buffered saline solutions has been reported to have some harmful physical effects (Golibersuch, 1973), few studies have correlated these physical effects with biological effects in detail. Many cell-biology experiments use buffered cell suspensions; this report details the biological and physical effects of suspending mouse myeloma cells in Dulbecco's phosphate-buffered saline (PBS) (Dulbecco and Vogt, 1954).

Because mouse myeloma cells grow in suspension culture, electrical (Coulter) counting to establish growth curves is particularly convenient and also quite accurate. The myeloma cell system allows multiple samplings from a single flask, in contrast to monolayer cell systems, which require parallel platings. This determination of a complete growth curve from a single suspension culture flask minimizes the error introduced by growth variation in parallel platings. Therefore, accurate and reproducible growth curves have been established by electrical counting of mouse myeloma cells (MacCoss et al., 1978; Griego et al., 1981).

The technique of cell counting by electrical resistance provides a number distribution of cell electrical size. Electrical counting relies upon the cell modifying an electrical field as it passes through a cylindrical aperture. The relative pulse height generated by a cell passing through the field is influenced by cell volume, resistivity, and shape. For cells of the same shape, variation in cell volume and/or resistivity is reflected by changes in the electrical size distribution (ESD). Previous investigations indicated that a change in electrical size might be an indication of cell death. Terasaki and Rich (1964) reported a decrease in cells of normal electrical size and an increase in cells of smaller electrical size after exposing mouse lymphocytes to antibody and complement. They believed that the increased number of cells showing a smaller ESD were dead cells with decreased resistivity due to increased cell membrane permeability. However, the electrical size instrument used in their study could generate an ESD only by a series of measurements. When the Coulter counter is equipped with a multichannel analyzer, an ESD can immediately be obtained as a single measurement and visually presented with an oscilloscope (Kubitschek, 1960; Adams et al., 1967). Because this methodology enables a rapid and complete determination of ESD, we used this improved technique to measure changes associated with saline treatment of mouse myeloma cells.

In our initial experiments using the Coulter counter, we observed results similar to Terasaki and Rich (1964) when mouse myeloma cells were suspended in PBS: a decrease in cells showing a larger ESD and an increase in cells showing a smaller ESD. A number of biological parameters indicated that PBS treatment was causing a significant amount of cell death. In addition, viability determined by measuring ESD of mixtures of live and dead cells showed excellent correlation with viability determined by standard methods. This paper establishes electrical sizing as an effective method for distinguishing live and dead mouse myeloma cells after PBS treatment, and also examines the

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physical parameters that determine this change in electrical size.

METHODS

Culture Techniques

Subclone 66.2 of mouse myeloma MPC11 cell line (Kuehl and Scharff, 1974), was grown in a suspension of Dulbecco's modified Eagle's medium, containing 20% heat-inactivated horse serum, 10% NCTC-109 medium, 1% nonessential amino acid solution (100X), 80 units/ml penicillin, 80 μg/ml streptomycin, and 3.3 mM L-glutamine, at 37°C in a water-saturated mixture of 5% CO_2 and 95% air (Kuehl and Scharff, 1974).

Treatment of Mouse Myeloma Cells with Isotonic Solutions

For cell lysis, cells were labeled (0.25 μC/ml) with ^{14}C-thymidine (ICN, specificity activity 60 mCi/mM) by growing cells exponentially for 24 h in culture medium. The cells were washed free of excess label by centrifugation (500 × g, 5 min, 18°C) and suspended in non-radioactive culture medium. The cell suspension was divided into three 10-ml fractions for electrical counting and radioactivity measurements. The three suspensions were centrifuged, and electrical counting and radioactivity measurements were performed on the supernatants. The cell pellets were suspended in culture medium, Dulbecco's phosphate buffered saline (PBS) (GIBCO Laboratory, Grand Island, NY, catalogue No. 310–4040), or PBS plus 1% bovine bovine serum albumin (PBS-BSA). Each solution was again centrifuged and resuspended in the appropriate solution. Radioactivity and electrical cell counts were then determined for the final suspensions. After 10 min at 37°, electrical counts and radioactivity were redetermined. Radioactivity was measured by liquid scintillation counting, the ^{14}C counts per minute (cpm) were 50% of the disintegrations per minute (dpm).

Electrical Sizing

The Coulter counter was a Model 55-1 Particle Counting System from Radiation Instrument Development Laboratory, Division of Nuclear Chicago Corporation and was used in conjunction with a Nuclear-Chicago Model 512 multichannel analyzer. The electrical sizing system consisted of a 100-μm diameter aperture (Coulter transducer), constant current generator/preamplifier, amplifier, a multichannel analyzer (127 channels), and oscilloscopes for displaying electrical size distributions and transducer signals. The preamplifier was set at 1.0 relative volume and X1 gain. The linear amplifier of the particulate counting system was used at a coarse gain setting of ⅛, a fine gain setting of 0.7 and the discriminator set at 0.0.

An aliquot (generally 0.1 ml) of cell suspension was placed in a plastic dilution vial and 10 ml of electrolyte was added. The electrolyte was PBS (Dulbecco and Vogt, 1954) with 0.04% formalin. After mixing by repeated inversions, readings were taken immediately. Electrical size signals from the Coulter transducer were accumulated for 0.1 min and subjected to pulse-height analysis. Conversion of electrical counts to cell number was accomplished by multiplying the electrical counts from an 0.1 ml sample by 830. This factor was obtained by calibrating electrical counts with light microscope counts of mouse myeloma cells in a hemacytometer.

Latex microspheres of 5.2, 8, 15, and 20 μm diameter were used for size calibration. The maxima of electrical size distribution profiles were plotted against the microsphere volume (Fig. 1), defining a linear relationship between channel number (electrical size) and geometric volume of the microsphere. Below channel number 9 (the maximum of the 8 μm microsphere ESD), resolution of the 5.2 μm microspheres was decreased, resulting in a nonlinear relationship (Fig. 1).

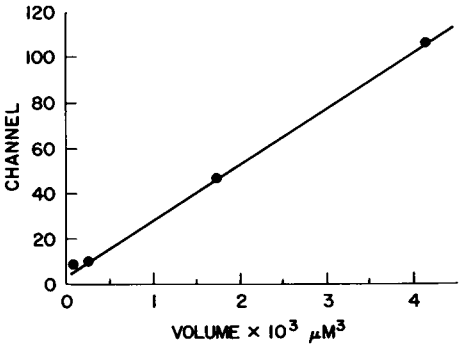

FIGURE 1 Calibration of multichannel analyzer with standard latex microspheres. The modal channel of the electrical size distribution is plotted against the geometric volume.

ESD Viability Determination

Two different, well-known methods were used to determine cell viability: exclusion of trypan blue and cloning on soft agar. In addition, viability was determined by electrical sizing. When PBS-killed cells were present the ESD were bimodal. The number of cells registering in the peak spanning the higher channels (i.e., those of larger electrical size) was taken as the number of viable cells. The number of dead cells was taken to be the difference between the total cell count and the viable cell count. The channel of minimum cell count between the two peaks was defined as the separation point between the two populations.

Trypan Blue Staining

A staining solution composed of 4 parts 0.2% trypan blue and 1 part 4.25% NaCl was made fresh daily. A conversion factor of 12.5×10^3 for a 1:5 dilution of cells with staining solutions was used to convert hemacytometer counts to the number of cells/ml.

Soft Agar Cloning

A 3.2% SeaKem agarose (FMC-Marine Colloids, Rockland, ME) water solution (20 ml) was autoclaved, and the hot agarose (7.5 ml) was immediately added to 100 ml of 50% conditioned medium (and 50% fresh growth medium) prewarmed for 10 min at 42°C. Conditioned medium was obtained by growing cells to 2–3 × 10^6 cells/ml, centrifuging, and then filtering the supernatant medium through a 0.22 μm filter (Millipore Corp, Bedford, MA). The agarose medium was incubated for at least 10 min longer at 42°C and dispensed in 4 ml aliquots into sterile pouring tubes. 0.1 ml of appropriate cell dilutions, determined by electrical counting, was added to each pouring tube and immediately poured into 6-cm plastic dishes. The agar was solidified by cooling (4°C, 5 min); the plates were then incubated at 37°C in the presence of water-saturated 5% CO_2, 95% air for one week. Cloning efficiencies of 15–30% were obtained.

Cell Killing Methods

Isotonic Buffer Suspension. (PBS Treatment). Exponentially growing cells (10 ml) were centrifuged for 5 min, 20°C, at maximum speed in an International (Damon Corp., Minneapolis, MN) clinical centrifuge. The cell pellet was resuspended in 10 ml of PBS and recentrifuged. The pellet was again suspended in 10 ml of PBS and incubated 10 min at 37°C. For the experiments described in Fig. 3, the cells were centrifuged, resuspended in 5 ml of 50% conditioned medium and mixed with various amounts of untreated exponentially growing cells. Parallel trypan blue staining and electrical sizing were then performed on the mixtures. All samples were stored in conditioned medium after treatment to minimize further cell death during processing and counting.

Light Microscope Sizing

Cells were suspended in trypan blue staining solution and placed on a hemacytometer. The cell diameters of 100–300 cells were measured using a microscope with an eyepiece micrometer (500X). The eyepiece micrometer was calibrated using latex microspheres.

Light Scattering Techniques

Exponentially growing mouse myeloma cells were centrifuged and resuspended in either PBS or culture media and incubated at 37°C for 1 h. Samples were then taken and light-scatter measurements of cell size were made using a Bio-Physics Cytograph model 6300A. Cells in suspension were individually illuminated by a milliwatt helium-neon laser. The scattered light, which is proportional to cell size, is sensed and the signals are processed by pulse analysis; the resulting distribution is stored on a multichannel analyzer.

RESULTS

Electrical counting and sizing measurements were performed in PBS containing 0.04% formalin (PBS-formalin) to minimize contamination of the counting apparatus. Electrical counts and sizing profiles were identical in both PBS and PBS-formalin counting solutions (data not shown). The electrical counts and sizing profiles did change when cells were allowed to remain in either counting solution for longer than several minutes. Therefore, all electrical measurement data presented in this paper are from samples measured within 15 s after suspension in counting solution.

PBS Exposure Lyses Mouse Myeloma Cells

The suspension of cells in PBS resulted in a reduction in the total number of recoverable cells after centrifugation (Table I, Fig. 2). This cell loss during centrifugation in PBS could be from cell lysis from PBS treatment, increased adherence to vessel walls, or cell clumping. Suspensions containing cells with radioactively labeled DNA would show increased radioactive label in the supernatant if cell lysis occurred, but would show decreased total radioactivity if cells adhered to vessel walls during centrifugation. Because the radioactive label in cells was almost completely recovered after two centrifugations (94%), the cell loss (41%) could not be because of adherence to vessel walls. Cell clumping was also not observed during PBS treatment by either electrical sizing or light microscopy. Therefore, cells were lysed during PBS treatment as indicated by the decrease in electrically countable cells (41%) and the increase of label appearing in the discarded supernatants (Table II). However, the amount of label found in the discarded supernatants (12.5%) did not account for all the lysed cells (41%). Because most of the

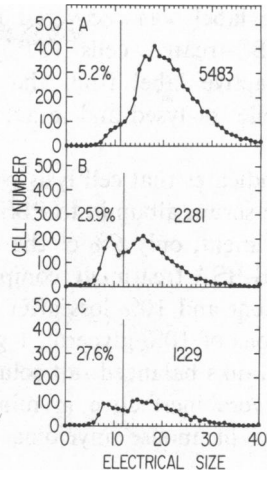

FIGURE 2 ESD of PBS-treated mouse myeloma cells. A parent culture (*A*) of exponentially growing cells was centrifuged and resuspended in PBS (*B*). After a second centrifugation and resuspension in PBS the cells were incubated for 10 min at 37°C, centrifuged, and resuspended in PBS (*C*). An identical volume (0.1 ml) of cell suspension was counted from all three samples. The channel numbers from the multichannel analyzer are proportional to electrical size. The electrical counts per channel are proportional to cell number per channel. Total electrical counts are given in these and other figures.

TABLE I
PBS-INDUCED CELL LOSS DETERMINED BY ELECTRICAL COUNTING

	(*a*) Growth medium treatment	(*b*) PBS treatment	%*b*/*a*
Parent culture	4,466 ± 176	—	—
After first centrifugation and suspension	4,451 ± 67	3,510 ± 59	78.9
After second centrifugation and suspension	3,950 ± 225	2,332 ± 195	59.0
After 10 min, 30°C; third centrifugation and suspension	3,183 ± 67	1,953 ± 140	61.4

Cells were treated with PBS and growth medium as described in Methods and five samples were counted electrically after each treatment step.
The mean is given along with the corresponding standard deviation.
The numerical values represent total electrical counts obtained from sampling a solution of 0.1 ml cell suspension in 10 ml counting fluid for 0.1 minutes.

TABLE II
DISTRIBUTION OF RADIOACTIVITY AFTER PBS TREATMENT OF MOUSE MYELOMA CELLS

	Cells/ml × 10^{-6}‡	Total supernatant radioactivity	Radioactivity in final cell suspension
		(cpm)	*(cpm)*
Parent culture	2.45		9,288,550
After culture* medium treatment	2.22	49,850	10,007,300
After PBS* treatment	1.44	1,053,800	7,643,250
After PBS-BSA* treatment	2.31	46,450	9,727,700

*See Methods for details of treatment.
‡Determined by electrical counting.
§Total supernatant radioactivity is the total cpm in the three supernatants discarded before the final suspension of the cell pellet. Electrical counts of the supernatant were background or lower values.

initial radioactive label was recovered in the final cell suspension of PBS-treated cells (82%), a significant amount of radioactive label from the lysed cells was trapped in the pellet of lysed and intact cells during the centrifugation.

Table II also indicates that cell lysis was minimized by adding 1% bovine serum albumin to PBS (PBS-BSA). In this typical experiment, only 6% of the electrical counts was lost after PBS-BSA treatment, compared to 41% loss after PBS treatment and 10% loss after culture medium treatment. Solutions of 10% glycerol, 1 g/l glucose, and another buffer (Hanks balanced salt solution; Hanks and Wallace, 1949) were ineffective in minimizing lysis of dilute suspensions of mouse myeloma cells (data not shown).

PBS Treatment Causes an Increase in Trypan-Blue Staining of the Remaining Cells

Another indication that mouse myeloma cells were seriously affected by dilution in PBS was the increased fraction of the remaining, unlysed cells that admitted trypan blue. Table III indicates that more cells admit trypan blue with increasing exposure to PBS by centrifugation or incubation at 37°C. Cells in growth medium showed little or no increase in trypan blue staining after centrifugation and incubation at 37°C.

PBS Treatment Causes a Decrease in the Capacity of Remaining Cells to Reproduce

A further indication that myeloma cells were affected by PBS was the decrease in the fraction of remaining cells able to form colonies on soft agar (Table III). After three centrifugations and suspensions and a short 37°C incubation in PBS, ~60–70% of the remaining unlysed cells could not reproduce to form colonies on soft agar. Cells undergoing the same treatment in culture medium (with serum) showed the same ability to form colonies as the parent culture.

PBS-treated Cells Showed a Smaller Electrical Size

A fraction of the mouse myeloma cells that had been exposed to PBS showed a significant change in electrical sizing profiles (Fig. 2). Cells sampled directly from the growth medium showed a bimodal distribution of electrical size, with 5–10% of the cells registering in channels 1–15 (<10 μm apparent diameter, Fig. 1) and 90–95% of the cells above channel 15. After PBS treatment, the number of cells registering in this lower channel peak increased significantly (Fig. 2*B* and *C*) and appeared to parallel the increase of trypan blue staining and nonreproducing cells (Table III).

TABLE III
SENSITIVITY OF MOUSE MYELOMA CELLS TO PBS TREATMENT

	% Trypan blue-admitting cells		% Reproductive cells	
	PBS treatment	Culture medium treatment	PBS treatment	Culture medium treatment
Parent culture	4	4	100*	100*
After first centrifugation and suspension	18	7	72	99
After second centrifugation and suspension	22	9	66	94
After 10 min, 37°C; Third centrifugation and suspension	37	9	36	107

*The parent culture was assumed to be 100% viable in soft agar cloning experiments. Other cloning percentages were obtained by dividing the number of sample clones by the number of parent culture clones and multiplying by 100.

Kinetics of PBS Effect on Mouse Myeloma Cells

The PBS effect was observed in every experiment, but the kinetics and degree of the effect varied from experiment to experiment. In Tables I, III, and IV, the maximal effect was observed after two centrifugations and a 10 min 37° incubation. Other experiments showed a maximal PBS effect after one centrifugation and resuspension (data not shown). Cells taken from the growth medium and suspended in the PBS counting solution show no PBS effect when suspended in the PBS counting solution and counted immediately on the Coulter counter. However, cells suspended in counting solution showed a gradual increase in the lower channel peak cells (dead and/or lysed cells) after several minutes. Therefore, the removal of the medium by centrifugation sensitized the cells to killing and lysis by PBS.

PBS-affected Cells Showed a Correlation of Smaller Electrical Size with Trypan-Blue Staining

The correlation between the population of cells stained by trypan blue and those of smaller electrical size was examined in detail. Both determinations were performed with a series of cell suspensions containing varying percentages of PBS-treated cells resuspended in growth medium with untreated cells. Trypan-blue stained and dye-excluding cells were counted, and parallel electrical counts and ESD analyses (Fig. 2) were performed. The fraction of smaller-sized cells determined electrically was plotted against the fraction determined by staining, and the line fitted by linear regression analysis (Fig. 3). The correlation between the two determinations was excellent (correlation coeffi-

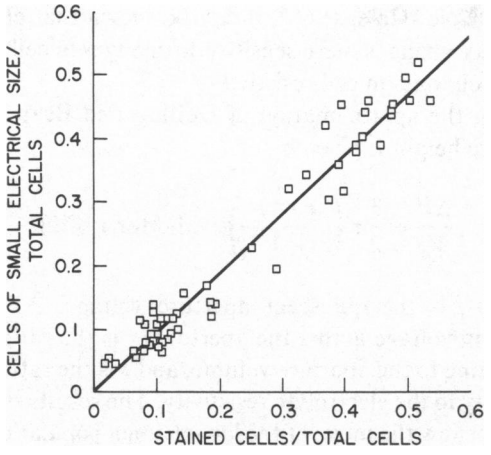

FIGURE 3 Correlation of the dead cell fraction determined electrically with that found by trypan-blue staining. Mixtures of PBS-killed cells and exponentially growing cells (see Methods) were counted under the light microscope after staining with trypan blue. The counts in the higher channel peak of the bimodal ESD were taken as the viable cell fraction, and the dead cell fraction was obtained by difference. The fraction of dead cells measured electrically (ordinate) is plotted against the fraction of dead cells that stained with trypan blue (abscissa). The correlation coefficient of the line, generated by linear regression analysis, is 0.98.

cient = 0.98) for these mixtures of untreated and PBS-treated myeloma cells.

PBS-Affected Cells Showed a Correlation of Trypan-Blue Staining with Smaller Physical Size

The diameter of PBS-treated cells was measured with the light microscope after trypan blue staining (see Methods). The parent cells and the intact PBS-treated cells both had round cross sections. Examination of PBS-treated cell suspensions showed mostly stained and unstained cells with some cell debris, nuclei, and large, ill-defined swollen structures. Only the intact stained and unstained cells were measured. The mean diameter for stained (dead) cells was smaller than that for unstained cells (Fig. 4). The size distribution of unstained PBS-treated cells was similar to that of the parent culture (data not shown). These data indicated that cells which were killed by PBS treatment decreased in geometric size as well as electrical size (Fig. 3).

PBS-affected Cells Showed a Correlation of Smaller Electrical Size with Decreased Light Scattering

Various samples were measured simultaneously by electrical counting and by light scattering (Table IV). The light scattering distribution (LSD) of parent culture cells (Fig. 5 *A*) was similar to the ESD of untreated mouse myeloma cells (Fig. 2 *A*) with a bimodal distribution of cells and a small number (~5–10%) of cells in the lower

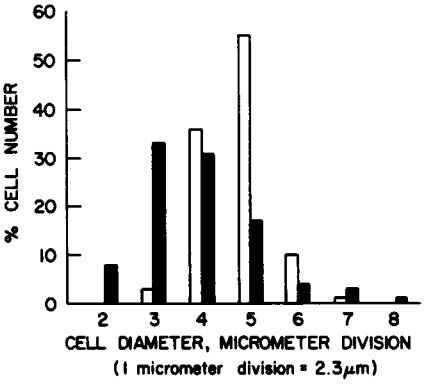

FIGURE 4 Histogram of the diameters of PBS-treated mouse myeloma cells (200–300 cells were counted) Cells were treated with PBS (see Methods and Fig. 2) and the size distributions of trypan-blue stained cells shaded) and dye-excluded cells (unshaded) were determined under the light microscope with an eyepiece micrometer. A micrometer division represents 2.3 μm.

channel (left) peak. PBS-treated cells also showed a similar effect in both electrical counting and light-scattering measurements; the left peak showed an increased proportion of cells in both light scattering (Fig. 5 *B*) and electrical counting (Fig. 2 *B* and *C*) after PBS treatment.

A time course of PBS treatment was determined by electrical counting and light scattering, and was compared to treatment with culture medium (M) and PBS-BSA (Table IV). Both methods show no increase in the number of small-sized cells after M and PBS-BSA treatment, but a marked increase of small-sized cells after PBS treatment. Although the correlation of small ESD cells with left peak LSD cells was excellent over the time-course study, ESD numbers were consistently higher than LSD numbers for the same sample.

TABLE IV
PBS-INDUCED DECREASE IN CELL SIZE DETERMINED BY BOTH ELECTRICAL COUNTING AND LIGHT SCATTERING

	Electrical counting*			Light scattering*		
		PBS-BSA	PBS	M	PBS-BSA	PBS
Parent culture	14.3	—	—	3.6	—	—
After first centrifugation and suspension	13.9	14.1	27.0	4.7	6.2	25.1
After second centrifugation, suspension, and incubation at 37°C						
min						
0	20.1	12.7	36.7	12.3	7.3	31.3
15	15.4	13.6	40.3	9.1	13.3	31.1
30	12.2	12.8	37.8	6.6	9.0	26.0
60	10.9	13.9	44.2	6.2	7.4	24.7

*Numbers are % of total cell counts found in the small-sized (left) peak in the bimodal distributions observed by electrical sizing (Fig. 2) and light scattering (Fig. 5).

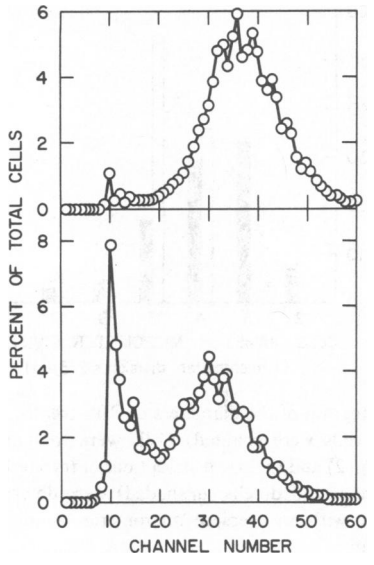

FIGURE 5 LSD of untreated and PBS-treated mouse myeloma cells. A parent culture (*top panel*) of exponentially growing cells was centrifuged and resuspended in PBS. After a second centrifugation and suspension in PBS, the cells were incubated for 15 min at 37°C (*bottom panel*). The channel numbers are proportional to the amount of light scattering. A total of 20,000 cells were counted for each light-scatter size distribution.

DISCUSSION

Counting cells by detecting the electrical pulses generated by cell passage through an electric field in an aperture is a well-established technique. The advantages of the electrical counting technique over laborious microscopic procedures are speed and accuracy. A major disadvantage has been the lack of discrimination in the method, that is, the inability to differentiate live cells from dead cells. This paper demonstrates that treatment of mouse myeloma cells with an isotonic saline solution (PBS) resulted in considerable cell death and lysis. Furthermore, these effects were detected by electrical counting and sizing techniques. This observation enabled the establishment of electrical counting and sizing as a convenient, effective method for distinguishing live and dead mouse myeloma cells after PBS treatment.

In considering the physical parameters that determine the smaller ESD of dead cells, we have assumed that both live and dead myeloma cells are essentially the same shape (spheroid). This assumption was verified by the observation of round cellular cross sections by light microscopy. The assumption that all cells have the same shape makes two physical parameters important for electrical resistance sizing: cell volume and cell resistivity. The decrease in pulse heights observed for a fraction of the cell population after serum-free buffer treatment can be attributed to either a decrease in cell volume, or a decrease in cell resistivity (an increase in membrane permeability), or some combination of these two factors. From the original analyses by Maxwell (Maxwell, 1892) or the approximation by Cole (Cole, 1968), it can be shown that electrical resistance sizing is more sensitive to changes in cell volume than to changes in cell resistivity.

Using the approximation of DeBlois and Bean (1970), the pulse height is given by

$$\frac{\Delta V}{V_o} = \frac{3}{2}\tau\left(\frac{r-1}{r+1/2}\right) \text{ (valid for } \tau \ll 1)$$

where V_o is the quiescent aperture voltage, ΔV is the change in voltage across the aperture, τ is the ratio of the cell volume to the aperture volume, and r is the ratio of cell resistivity to the electrolyte resistivity. The results shown in Fig. 2 *B* show the mode of the low-channel population to be at channel 9, while the mode of the untreated cells (Fig. 2 *A*) is at channel 18. Using the subscript d to indicate the low-channel population, and ℓ the untreated cells: $(\Delta V/V_o)_d \simeq \frac{1}{2}(\Delta V/V_o)_\ell$. If the change in pulse height were due only to a change in cell volume, then $\tau_d \simeq (1/2)\,\tau_\ell$. Assuming that the model diameter of untreated myeloma cells is 10 μm, one may calculate that the modal diameter of cells with one-half the volume of 10 μm sphere is ~8 μm. The results of sizing PBS-treated cells by light microscopy (Fig. 4) show a difference in modal diameters for trypan-blue staining cells vs. dye-excluding cells, in agreement with this calculation. The results of the light-scatter sizing (Fig. 4) also suggest that the PBS-treated cells include a population of smaller sized cells.

The alternative possibility is that the decreased pulse heights of the PBS-treated cells were caused solely by an altered cell resistivity:

$$\left(\frac{r-1}{r+1/2}\right)_d \simeq \frac{1}{2}\left(\frac{r-1}{r+1/2}\right)_\ell.$$

Substituting into this equation the approximation (Cole, 1968) $r = (\rho_2 + \rho_{mem/a})/\rho_1$ where ρ_{mem} is the membrane impedance, a is the cell radius, and ρ_2 and ρ_1 are the internal and external electrolyte resistivities, letting $\rho_2 = \rho_1$ and assuming $(\rho_{mem})_\ell$ is infinite, leads to $(\rho_{mem})_d \simeq 3.75 \times 10^{-2}\ \Omega\text{cm}^2$. This is a low value of membrane resistivity; for comparison, ρ_{mem} for human erythrocytes has been reported as 10 Ωcm^2 (Johnson and Woodbury, 1964). However, it is not possible based on the evidence presented in this paper to exclude decreased membrane resistivity as an effect of PBS treatment of mouse myeloma cells. Geometric size is the major determinant in light scattering, but large changes in membrane permeability, cell color, or cell transparency can alter the LSD (Crissman et al., 1975). Therefore, a complete resolution of this question must use other techniques. Based on the light microscope sizing results, decreased cell size is an effect of PBS treatment.

Although there was a correlation of trypan-blue stained cells with the peak of smaller ESD (Fig. 3), some cell

debris and nuclei might also be counted in this peak. The least-squares linear regression line generated by the data in Fig. 3, argues somewhat against this possibility because the slope of the line was 0.98; significant counting of cell debris and nuclei in the smaller ESD peak would cause the slope to increase to >1.0, because these objects were ignored in the determination of abscissa values. However, the amount of cells observed in the smaller LSD peak was significantly lower than the amount observed in the smaller ESD peak after PBS treatment (Table IV). These data suggest that the Coulter Counter (ESD) was detecting additional particles other than cells in the left peak that were not detected by light scattering.

The correlation of an increased number of dead cells with an increase of cells of smaller electrical size (Fig. 3), agrees with the previously published work of Terasaki and Rich (1964). They showed an increase in cells of smaller electrical size after exposing mouse lymphocytes to antibody and complement. They also concluded these dead cells had decreased resistivity due to an increase in cell membrane permeability. In this experiment, mouse myeloma cells were killed with PBS treatment, as shown by several biological determinants of viability, including trypan-blue staining and growth in soft agar. In contrast to the conclusion of Terasaki and Rich, however, our results from light-scatter sizing and optical microscopy suggest decreased geometric size as the major factor in reduced electrical size distribution for cells killed by PBS treatment. The different conclusions may be due to the difference in cell systems or cell killing methods.

The ability to differentiate live and dead cells by electrical sizing gives the electrical counting technique an even greater advantage over microscopic procedures in both convenience and accuracy of counting cells in buffer suspensions. In addition, we have reported an effective way of minimizing PBS-induced death by using BSA (Tables II and IV). Smaller ESD have also been observed in myeloma cells killed with cytosine arabinoside, 8-azaguanine, methyl methanesulfonate, and ultraviolet irradiation (data not shown). Therefore, electrical sizing could potentially be used to discriminate between live and dead cells under many other conditions besides PBS treatment. However, a careful correlation of ESD changes with cell viability must be established with many agents and different cell lines to make this method more generally useful.

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