COMPARISON OF COULTER VOLUMES WITH RADIOMETRICALLY DETERMINED INTRACELLULAR WATER VOLUMES FOR CULTURED CELLS

NEAL S. BURRES1 AND CAROL E. CASS2

McEachern Laboratory and Department of Biochemistry, University of Alberta, Edmonton Alberta, Canada T6G 2H7

(Received 26 September 1988; accepted 3 January 1989)

SUMMARY

During methotrexate-induced differentiation of cultured human choriocarcinoma (BeWo) cells, proliferation is inhibited, morphologic and biochemical changes occur, and giant, often multinucleated, cells form. We have used the increase in cell volume as a marker of the mature syncytiotrophoblastlike phenotype. Uninduced and differentiated BeWo cells are not spherical, and theoretical considerations suggested that deviations in shape could result in significant errors in Coulter volume. To determine if the values obtained by electrical pulse sizing reflected the actual mass of BeWo cells, we have evaluated the relationship between Coulter volumes and intracellular water volumes obtained using a shape-independent estimate for eight cell types. A close correlation ($r^2 = 0.97$) was found, indicating that cell volume changes in populations of irregularly shaped cells can be accurately measured using a Coulter instrument.

Key words: BeWo cells; cell volume; Coulter principle.

INTRODUCTION

Electrical resistance pulse sizing, discovered by Coulter in 1953 (8), is based on the principle that particles suspended in an electrolyte contribute to the net resistivity of the compound medium (21). Although the resistance of a suspension was initially thought to be independent of cell shape (19), this consideration proved inadequate when it was shown that differently shaped particles of equal volume did not have the same resistance in a model of the orifice of a Coulter instrument (12). Maxwell's (21) equation describing the resistance of a compound medium for a single particle predicts that variations in cell resistivity, shape, orientation, and the applied current can alter the change in voltage observed when a particle migrates through the electrical field in the aperture of a resistance pulse-sizing instrument (9,12,13,17,18,23). Although electrical determination of cell volume is commonly used, the effect of cell shape is not often considered (for review, see ref. 18). Coulter instruments are routinely calibrated with plastic microspheres, which do not possess the characteristics of cells (requirement for isotonicity, deformability, irregular shape, nonhomogenous resistivity), and cell volumes may thus be subject to artifactual error.

While characterizing the large increases in cell mass occurring during methotrexate-induced differentiation of human choriocarcinoma (BeWo) cells (4,5), it became apparent that the cells used for volume determinations were irregularly shaped. The objective of the present study was to assess the fidelity of electrical resistance pulse sizing of nonspherical cells. There was a close correlation for BeWo cells and seven other cell types between Coulter volume measurements and intracellular water volume, which was determined with a shape-independent, radiochemical method.

MATERIALS AND METHODS

Cultured cells were maintained at 37°C in a humidified atmosphere of 5% CO$_2$ in air. Stock cultures of all continuous cell lines were grown in antibiotic-free growth media and were free of Mycoplasma. Adherent cell lines were subcultured weekly by disassociation with trypsin-EDTA (0.05% trypsin, 0.02% EDTA, in 0.15 M NaCl). Suspension cultures were maintained at population densities of less than 5 x 10$^5$ cells/ml by dilution in fresh growth media at 2- to 3-d intervals. Cell numbers were determined with a Coulter counter (model Zf, Coulter Electronics, Hialeah, FL). Unless otherwise indicated, cultures used to provide cells for volume determinations were initiated in growth media containing penicillin (100 U/ml) and streptomycin (100 μg/ml) and were harvested in midphase of exponential growth. Cell culture materials were purchased from Grand Island.

1 Present address: Harbor Branch Oceanographic Institution, 5600 Old Dixie Highway, Ft. Pierce, Florida 33450.
2 To whom requests for reprints should be addressed.
Biological Co. (Burlington, Ontario) and Flow Laboratories (Mississauga, Ontario).

Human choriocarcinoma (BeWo) cells, obtained from Dr. S. J. Friedman, University of Calgary, were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (4,5). Cultures were initiated with 2 x 10^6 cells/T-25 flask in 5 ml growth media, and for induction of cellular differentiation culture fluids were removed after 24 h and replaced with fresh growth media containing 1 μM methotrexate (4,5). For volume determinations, cultures were harvested by trypsinization 72 h after establishment.

Human cervical carcinoma (HeLa/S3) cells, obtained from American Type Culture Collection, Rockville, MD, were maintained in Eagle’s minimal essential medium supplemented with 10% calf serum (6). For volume determinations, cultures were initiated with 10^6 cells/T-25 flask in 10 ml of growth media and were harvested by trypsinization 72 h later.

The origin of murine leukemia (L1210/C2) cells has been described elsewhere (7). L1210/C2 cells were maintained in suspension culture in Fischer’s medium supplemented with 10% horse serum. For volume determinations, cultures were initiated with 10^6 cells/ml (10 ml total) in 60-ml glass bottles and were used 36 h later. Rat Novikoff hepatoma cells, obtained from Dr. P. W. G. Plagemann, University of Minnesota, were maintained in Eagle’s minimal essential medium supplemented with 5% horse serum, 0.1 mM nonessential amino acids, and 0.1 mM glutamine (11). For volume determinations, cultures were used at a density of less than 4 x 10^6 cells/ml.

Peripheral blood from healthy human volunteers was collected into 10-m1 heparinized (143 U.S.P. U/tube) Vacutainer tubes (Becton Dickinson, Rutherford, NJ). The volume of erythrocytes was obtained after diluting whole blood that had been incubated 45 min at 37°C in the presence of 0.5% carbonyl iron and gum arabic (Sigma Chemical Co., St. Louis, MO) by centrifugation (400 xg, 10 min) over 60% Percoll in 0.15 M NaCl, as described previously (1). Leukemic myeloblasts were isolated from the whole blood of a patient with acute myelogenous leukemia by centrifugation (400 xg, 10 min) over 60% Percoll in 0.15 NaCl (11).

To electrically determine cell volumes, cells were diluted in 0.15 M NaCl, and volume histograms were obtained with a model Zf Coulter counter (100 μM aperture tube) in conjunction with a 100-channel particle size analyzer (Channelyzer II, Coulter Electronics, ed circuit was on). The channel analyzer was calibrated with polystyrene spheres of 10.08 μM diameter obtained from Coulter Electronics. All voltage pulses were accumulated at a coincidence rate of less than 5% until the model peak totaled 1000 cells. Volume histograms were analyzed with an Apple II+ computer (Apple Computer Inc., Cupertino, CA) with software obtained from Coulter Electronics and in all cases the average mean and standard deviations of several histograms have been reported. A shape factor of 3/2, corresponding to a nonconducting spherical particle, was used for all determinations (13,21). BeWo cells and erythrocytes were irregularly shaped, whereas the other cell types did not significantly differ in shape from that of a sphere, as determined by microscopic analysis (final magnification = x250). The relationship between voltage pulse height and particle volume was determined with microspheres of mean diameters of 5, 9.54, 9.69, 10.08, 10.14, 15, and 25.7 μM (Coulter Electronics; Duke Standards, Palo Alto, CA; Sigma Chemical Co.). A linear relationship (r^2 = 0.99) was found over this range.

Cell water volume was determined by adaptation of a previously described centrifugal method (14). Cultured cells were suspended in the appropriate growth medium, and blood cells were suspended in RPMI 1640 medium supplemented with 10% fetal bovine serum. Triplicate (duplicate for L1210/C2 cells) assay mixtures were prepared in 1.5-ml polystyrene microcentrifuge tubes (Bio-Rad Laboratories, Mississauga, Ontario). Added first was 150 μl of oil (8.49 parts Dow Corning 550 silicone oil and 15.1 parts Fisher 0-119 light paraffin oil; specific gravity of mixture, 1.03 ml) upon which was layered 100 μl of growth medium containing [3H]water (2 μCi/ml) or [14C]-sucrose (10 μCi/ml). [3H]Water (100 mCi/ml) and [14C]-sucrose (250 mCi/ml) were purchased from ICN Chemical and Radioisotope Division, Irvine, CA. Reactions were initiated by addition of 100 μl of growth medium containing 1 x 10^6 cells and were ended immediately thereafter by centrifugation for 30 s (14). The media portions of the incubation mixtures were removed by suction, leaving an oil layer over the cells, tubes were rinsed with 1.0 ml water, and 200 μl of 5% Triton X-100 was added to each tube. After incubating overnight, the tubes were transferred to counting vials and 10 ml of scintillation fluor was added (22). The intracellular water volume is defined as the total volume of the cell pellet (i.e. the [3H]water volume) minus the extracellular volume of the pellet (i.e. the [14C]-sucrose volume). Extracellular water volume was less than 10% of the total volume in all experiments.

BeWo cell diameters were optically determined by mixing trypsinized cells in a 1:1 numerical ratio with microspheres (nominal diameter of 9.54 μM as reported by manufacturer) before wet mounts were made. Photomicrographs were taken and the diameters of beads and the major and minor diameters of BeWo cells were measured directly with a vernier micrometer. The diameters of BeWo cells were calculated from the ratio of cell to bead diameter and the final magnification (x250).

RESULTS AND DISCUSSION

During exposure to methotrexate, BeWo cells stop proliferating and undergo a complex differentiative response that resembles in vitro development of syncytiotrophoblast (2-5, 10). After 48-h exposures to drugs, BeWo cultures are predominantly (>90%) populated with giant, often multinucleated, syncytiotrophoblastlike cells that display elevated levels of chorionic gonadotropin and placental alkaline phosphatase (2-5,10,16). While characterizing the increases in cell mass accompanying...