Enumeration of Primary Tissue Cells by an Electronic Device

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Cell yields from primary tissue suspensions of chicken embryos, rhesus, and cercopithecus monkey kidneys, were compared using the hemocytometer and Coulter counter, to determine the suitability of the electronic device for routine tissue culture practices. Plating efficiencies indicated that the Coulter counter is equal to or better than the hemocytometer for enumeration purposes. Results with the counter were more consistent and were reproducible, and there was a twofold saving in time, as well as elimination of the necessity of visual counts. The electronic counter has been used routinely in the laboratory for the past year, and in all instances, suitable monolayers for tissue culture production have been obtained.

A SIMPLE, quick, and reproducible method for determining cell yield from primary tissue has become a necessity for large scale tissue culture production. Many methods of estimating mammalian cell populations have been reported (1, 2) but the hemocytometer and the index-of-viability dyes, *e.g.*, trypan blue (3, 4) and eosin (5, 6), have been the most widely used means of determining viable cell concentrations. However, hemocytometer counts have proved time consuming and subject to serious error (7, 8), and the dye-exclusion tests are not always reliable indicators of cell cultivability (9).

This laboratory utilizes an electronic counter[1] which enumerates individual cells by a form of electronic "gating," for establishing cell inoculum. The instrument has been evaluated for blood cell counting (10, 11), and its application in growth studies has been demonstrated (12). The purpose of this paper is to compare the data obtained with both the hemocytometer and the electronic counter, and to determine the suitability of the instrument for routine tissue culture practices. The evaluation scheme closely paralleled that described by Brecher *et al.* (10).

EXPERIMENTAL

Culture Methods.—Primary cell suspensions were prepared from freshly harvested chicken embryos, rhesus, and cercopithecus monkey kidneys. Following trypsinization (1, 13, 14) the cells were resuspended in appropriate growth media, thoroughly mixed, and samples were removed for enumeration. The remaining cell suspension was diluted, inoculated into culture bottles, and incubated at 36°. Confluence monolayers developed in 16–18 hr. for chicken embryo cultures and 7 days for monkey kidney cultures.

Enumeration.—Enumeration with the electronic counter is accomplished by a form of electronic "gating." As a particle is drawn through a small aperture (100 μ), an equal amount of electrolyte is displaced causing a voltage drop due to an increase in aperture impedance. The resultant pulses are amplified, recorded by a decade counter, and visualized on an oscilloscope (10–12). For the authors' use, the instrument was calibrated with ragweed pollen according to the method described by Coulter Electronics.[2]

For monkey kidney cells the optimum threshold setting on model A was found to be 20 with aperture current setting of 3 and a gain of 2, whereas for chicken embryo cells settings were 5, 2, and 5, respectively. Cells were suspended in phosphate-buffered saline (15) and 4 successive counts were taken on each sample, representing 4 aliquots of the same cell population, or a total of 2 ml.

Hemocytometer.—Hemocytometer counts were made on samples composed of equal parts of cell suspension and 0.5% trypan blue. The viable cells in 2 squares (2 sq. mm.) were counted and multiplied by a dilution factor of 10,000 to obtain total cells per milliliter. This, therefore, represents a sampling of $^1/_{10,000}$ of 1 ml. Dead cells were also enumerated and a percentage of the total determined on each count.

Reproducibility Techniques.—Both systems were further evaluated by comparing multiple counts on the same primary cell suspension. Five different suspensions of rhesus monkey cells were enumerated with at least 20 counts obtained on each suspension with each method. In all instances, the cell suspensions were slowly mixed on a magnetic stirrer, and samples were removed and enumerated by both methods within a 2-hr. period (10). The mean cell counts obtained from the 5 monkey suspensions were compared, and the standard deviations and standard errors were determined.

Routinely, over a 6-month period, 3 types of primary cell suspensions—chicken embryo, rhesus, and cercopithecus monkey kidneys—were subjected to comparison counts with the hemocytometer and the electronic counter. Representative samples were taken of each suspension and single counts were obtained. The data for each tissue (Fig. 1) were plotted and fitted to a regression line (16) to determine the association existing between both procedures in evaluating a primary cell system.

A modified plating-efficiency technique was applied to both enumerating systems. A primary

Received October 20, 1965, from the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.
Accepted for publication January 28, 1966.

[1] Marketed by Coulter Industrial Sales Co., Division of Coulter Electronics, Inc., Chicago, Ill.

[2] Coulter counter for particle content and size distribution, Coulter Electronics, 1958.

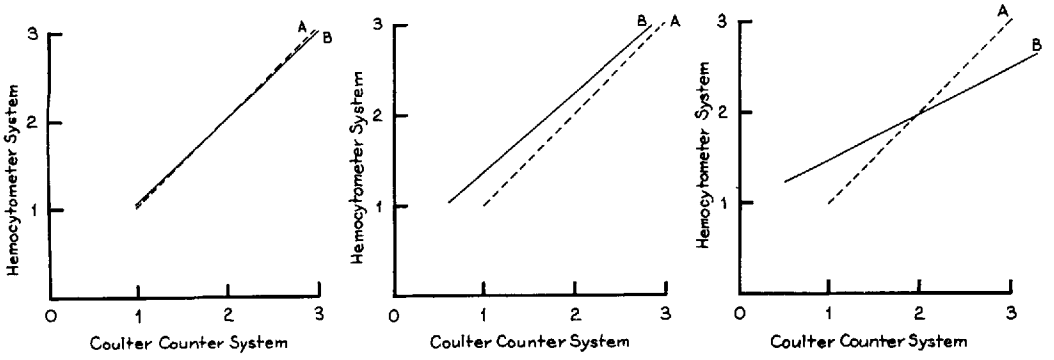

Fig. 1.—Relationship between hemocytometer and Coulter systems in evaluating primary tissue suspensions. Key: A, theoretical; B, Coulter counts plotted against hemocytometer counts. Counts are expressed in millions of cells per milliliter of suspension. Left: chicken embryo; middle: rhesus monkey kidney; right: cercopithecus monkey kidney.

suspension of rhesus monkey kidney cells was enumerated and, from this, two series of cultures were planted as determined from the counts of both systems. Each series consisted of two 2-oz. bottles at concentrations of 6,000, 12,000, 25,000, 50,000, 100,000, 200,000, and 300,000 cells/ml. Cell monolayer development was observed daily for 10 days.

RESULTS

The pooled standard deviation for the electronic method was 8.42×10^4 cells/ml. as compared with a standard deviation of 18.31×10^4 cells/ml. for the hemocytometer (Table I). The variability of each enumerating system on 5 separate monkey kidney suspensions is illustrated in Fig. 2 which represents that portion (95%) of the counted cells which lie in the interval of + or − 2 standard deviations. The standard errors for the electronic counter were all less than 2.4 while hemocytometer standard errors all exceeded 3.3 (Table I).

The data obtained from the routine comparison of primary cell suspensions provided correlation coefficients of 0.82 (chicken embryo), 0.76 (rhesus monkey kidney), and 0.70 (cercopithecus monkey kidney) which demonstrates the favorable relationship existing between both enumerating systems. The regression lines (Fig. 1) indicate the probability of a linear relationship.

Differentiation between counting systems was observed at a planting rate of 2.5×10^4 cells/ml. The cell cultures prepared from cells enumerated by the Coulter method had moderate growth in 10 days (40% confluency) as compared with 10–15% confluency in hemocytometer-enumerated cell cultures. No significant growth was noted at cell concentrations of 6000 and 12,000 cells/ml., whereas confluent monolayers were observed in 6 days at a concentration of 50,000 cells/ml. and in 4 days at concentrations of 100,000, 200,000, and 300,000 cells/ml.

DISCUSSION

The high degree of variation existing between mean hemocytometer and electronic counts has been demonstrated (Table I). It was shown from the 5 separate monkey kidney suspensions that the standard deviation for the electronic counter varied between 6.35–10.58 $\times 10^4$ cells/ml., whereas the standard deviation for the hemocytometer counts varied between 14.72 and 21.77 $\times 10^4$ cells/ml. The pooled standard deviation for the hemocytometer was more than twice that of the electronic counter. The standard errors agree well with those reported by Brecher (10), with the electronic counter errors all less than 2.4, while those of the hemocytometer all exceed 3.3.

The routine analysis of comparison counts provided correlation coefficients which demonstrate that both systems are fairly comparable. The coefficients are lower than those reported by Harris (12), probably because primary suspensions contain numerous cell types, blood, and debris. Correlation of hemocytometer and electronic counts was very good for chicken embryo tissue (Fig. 1, left), but to a lesser degree for simian tissue (Fig. 1, middle and right). In this study the cause of discrepancy was not determined, but the postulation of Mattern

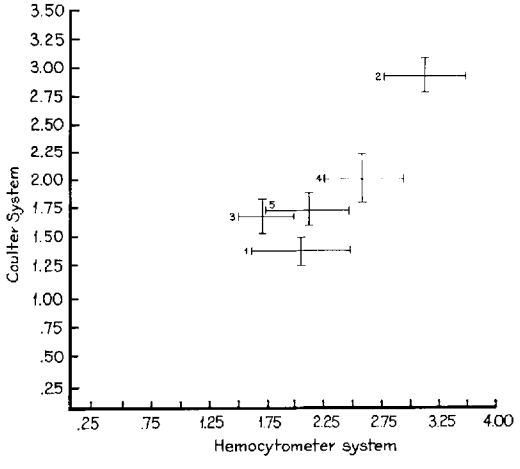

Fig. 2.—System reproducibility. Variation is expressed in terms of + or − two standard deviations from established mean cell count. Each kidney cell suspension is numerically indicated. Counts are expressed in millions of primary rhesus cells per milliliter.

TABLE I.—COMPARISON AND REPRODUCIBILITY OF ENUMERATING SYSTEMS (PRIMARY RHESUS KIDNEY CELLS)

Monkey Suspension	Method	Mean Count × 10^4/ml.	Dev.	Error
1	Hemocytometer	200.30	21.77	4.87
	Electronic[a] counter	136.55	6.35	1.41
2	Hemocytometer	310.20	17.90	4.02
	Electronic counter	290.95	8.02	1.79
3	Hemocytometer	165.55	14.72	3.29
	Electronic counter	169.00	9.29	2.08
4	Hemocytometer	256.00	17.76	3.97
	Electronic counter	203.35	10.58	2.37
5	Hemocytometer	207.88	18.63	3.73
	Electronic counter	174.44	7.51	1.50
Pooled	Hemocytometer		18.31	
	Electronic counter		8.42	

[a] Coulter counter.

et al. (11), that errors in hemocytometry must be involved, is accepted.

The plating efficiencies indicate that the electronic counter is equal to or better than the hemocytometer for determining cell inoculum. At a planting concentration of 2.5×10^4 cells/ml. a slightly higher per cent confluency was noted in the Coulter cultures.

From the data collected, it was noted that the number of nonviable cells from any one of the 3 cell suspensions never exceeded 10% of the total. Therefore, it must be assumed that the problem of variation in cell viability was overcome by the use of a standardized system of tissue culture preparation that consistently yielded a uniform population of viable cells.

This study indicates that the electronic counter is suitable for routine tissue culture practices, since it is more consistent than the hemocytometer and possesses excellent reproducibility. It is evident that the degree of variation existing in hemocytometry could eventually be responsible for the establishment of erroneous planting rates causing either a decrease or increase in the number of total cells available, which would directly affect the number of cultures obtained from a primary suspension. The utility of the electronic counter for routine operation is further supported by a twofold saving in time and the elimination of many stress factors that may contribute to erroneous visual counts. The electronic counter has been in operation in this laboratory for the past year as the standard enumerating method. During this period, 400 cercopithecus monkey kidneys, 220 rhesus monkey kidneys, and 61 lots of chicken embryo (approximately 206 embryos/lot) were trypsinized, planted, and in all instances gave rise to suitable monolayers for tissue culture production.

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