continuous nature of activity or concentration of species across the interface and extension of analysis to steady states.

The assumption (*e*) of paper I, which states that the mobilities of ions in surrounding solution are infinite, which enables the author to conclude that $\Gamma^{\pm} = 0$, is not reasonable since surrounding solutions have finite conductance.

Since none of the above-mentioned difficulties are insurmountable, the initial task should be to understand equilibrium state of inhomogeneous fluid boundary interfaces before analyzing transport processes in these systems in order to preserve consistency.

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An Effect of Cell Shape on Apparent Volume as Determined with A Coulter Aperture

Dear Sir:

The Coulter counter (Coulter, 1956) has been used extensively for the determination of total concentration of cells in suspension culture, as well as for the determination of volume distribution spectra. Gregg and Steidley (1965) have studied the relationship between particle volume and Coulter pulse amplitude and have shown that particle shape affects the apparent volume as determined from the increase in aperture resistance. We have recently observed anomalies in the determination of both cell number and cell volume for synchronized mammalian cells in suspension culture caused by failure of daughter cells to separate after mitosis is complete. That this leads to a low value of cell concentration is obvious; the effect on the volume spectrum is more subtle.

Chinese hamster ovary (CHO) cells were selectively removed from glass-grown monolayer cultures by a modification of the method of Robbins and Marcus (1964), using a mechanical shaking machine (Tobey, Anderson, and Petersen, 1967). The detached cells (shaken off in low calcium F-10 medium) were set up as suspension cultures in spinner flasks and were studied during the ensuing division wave.

The selected populations were initially almost entirely mitotic (mitotic fractions ranging from 0.85–0.98) and were very tightly synchronized, as evidenced by an abrupt drop of the mitotic fraction to less than 0.05 during the first 20 min in suspension culture. Total cell count rose much more slowly and depended strongly on the intensity of mixing (repeated pipetting) to which the suspension was subjected. The earliest separation of daughter cells which could be obtained was about 10 min after completion of the mitotic wave—a measure of the minimum temporal difference between the dissolution of the mitotic apparatus and cell separation, fiducial marks in cell life cycle analysis (Tobey et al. 1966). With minimal pipetting, a population could be obtained with over 80% of the cells as unseparated pairs (as determined by visual scoring of microscopic fields) for periods of some 40 min following mitosis.

Cell volume distribution spectra were measured during this period using a 90 × 200 mi-

cron Coulter aperture with cell transit time adjusted to match the electronic response of the amplifier and analyzer (Kubitschek, 1962). The resulting spectra are shown in Fig. 1 for times ranging from 0–70 min after shaking from monolayer. The initial population (curve *a*, mitotic fraction 0.85) shows a single peak of undivided cells with a modal volume of 2.0 (arbitrary units of pulse height), and the population at 70 min (curve *e*, mitotic fraction 0.03; visual fraction undivided 0.30) shows the peak of divided cells with exactly one-half the initial volume. At intermediate times the modal volume of the daughter peak is invariant, while that of the parent peak decreases to 1.64 (curves *c*, *d*, and *e*). The time at which the decrease

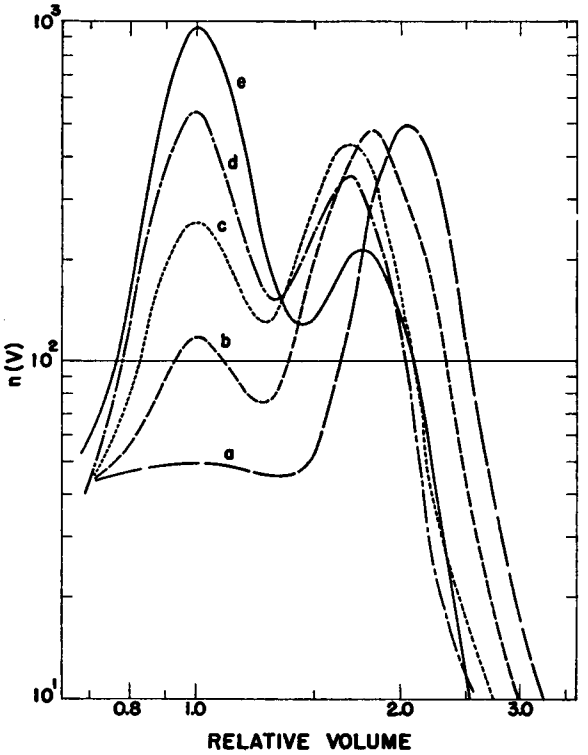

FIGURE 1 Cell volume distribution spectra for a synchronized culture of Chinese hamster ovary cells as it passed through cell division. The ordinate, $n(V)$, is the number of cells of volume V. The curves were measured at the following times after synchronization by shaking from monolayer: *a*, 0 time; *b*, 15 min; *c*, 25 min; *d*, 45 min; and *e*, 70 min.

occurs corresponds to the time of completion of mitosis, and the cells giving the apparent reduction in volume are those visually identifiable as pairs of tangent spheres. That the effect is not due to a true volume change is indicated by the constancy of the volume of the resulting daughter cells when separation finally occurs. Thus the apparent depression in volume is a result of the nonspherical shape of these cell pairs. The expected ratio of apparent volume-to-true volume can be estimated using equation 13 of Gregg and Steidley (1965), approximating the tangent spheres with a cylinder of the same ratio of diameter to length. The result of 0.82 is in excellent agreement with observation.

While the conditions of measurement reported here were such as to maximize the effect (tightly synchronized population and minimal mixing), it is clear that the sensitivity of the Coulter system to particle shape constitutes a potential source of significant error for the measurement of volume distribution spectra, even for such nominally "spherical" objects as mammalian cells in suspension culture. In applications requiring an accurate determination of the detailed shape of the entire volume spectrum [e.g., for estimation of volume growth rates and division probabilities (Bell and Anderson, 1967)], experimental conditions must be developed to minimize this source of error by ensuring complete dissociation of pairs of daughter cells. We have found that pipetting through small apertures (a few tenths millimeter diameter) or 10 min digestion with trypsin are effective, but visual determination of unseparated pairs is recommended as a routine control measure.

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Samuel A. Talbot, 1903–1967

Samuel A. Talbot died on 20 February 1967 in Baltimore at the age of 63. He was one of the pioneers in the areas of biophysics, medical physics and biomedical engineering. He received his Bachelor's degree from Cornell, his Master's degree from Trinity College, and his Ph.D. degree in physics at Harvard in 1935. His doctoral thesis was of a biophysical nature and probably one of the early ones for which a Ph.D. degree was awarded. Dr. Talbot then joined the faculty of The Johns Hopkins School of Medicine to continue work in physiological optics which he had already begun in his doctoral thesis. Eventually he was appointed chairman of a Biophysical Division and later chairman of the Department of Biomedical Engineering within the larger Department of Medicine at Johns Hopkins. In 1966, he was once more organizing a Biomedical Engineering Department at the University of Alabama when he fell ill.