METHODS IN CELL PHYSIOLOGY

Volume III

David M. Prescott

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VOLUME III

Contributors to This Volume

E. C. Anderson

Emil Bernstein

I. L. Cameron

D. Davidson

R. J. Harvey

D. F. Hülser

John Mendelsohn

D. S. Nachtwey

D. F. Petersen

James L. Pipkin, Jr.

M. F. Rajewsky

Howard Rothstein

Donald E. Rounds

Norman P. Salzman

Takeshi Seto

Jane L. Showacre

Gordon E. Stone

Elton Stubblefield

R. A. Tobey

Jack Van't Hof

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DAVID M. PRESCOTT

INSTITUTE FOR DEVELOPMENTAL BIOLOGY
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LIST OF CONTRIBUTORS

Numbers in parentheses indicate the pages on which the authors' contributions begin.

E. C. ANDERSON, Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico (347)

EMIL BERNSTEIN,[1] Department of Zoology, University of Maryland, College Park, Maryland (119)

I. L. CAMERON,[2] Department of Anatomy, State University of New York Upstate Medical Center, Syracuse, New York (213, 261)

D. DAVIDSON, Department of Biology, Case Western Reserve University, Cleveland, Ohio (171)

R. J. HARVEY, Department of Biology, State University of New York at Buffalo, Buffalo, New York (1)

D. F. HÜLSER,[3] Max-Planck-Institut für Biophysik, Frankfurt/Main, Germany (293)

JOHN MENDELSOHN,[4] Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland (277)

D. S. NACHTWEY,[5] Cellular Radiobiology Branch, U.S. Naval Radiological Defense Laboratory, San Francisco, California (213)

D. F. PETERSEN, Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico (347)

JAMES L. PIPKIN, JR. Department of Botany, University of Texas, Austin, Texas (307)

M. F. RAJEWSKY,[3] Max-Planck-Institut für Biophysik, Frankfurt/Main, Germany (293)

HOWARD ROTHSTEIN, Department of Zoology, University of Vermont, Burlington, Vermont (45)

DONALD E. ROUNDS, Pasadena Foundation for Medical Research, Pasadena, California (75)

NORMAN P. SALZMAN, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland (277)

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[1] *Present address:* Gillette Research Institute, Inc., Biomedical Sciences Division, Washington, D.C.

[2] *Present address:* Department of Anatomy, University of Texas Medical School at San Antonio, San Antonio, Texas

[3] *Present address:* Abteilung für Physikalische Biologie, Max-Planck-Institut für Virusforschung, Tübingen, Germany

[4] *Present address:* Peter Bent Brigham Hospital, Boston, Massachusetts

[5] *Present address:* Radiation Center, Oregon State University, Corvallis, Oregon

TAKESHI SETO,[6] Department of Biology, Kwansei Gakuin University, Nishinomiya, Japan (75)

JANE L. SHOWACRE, Institute for Developmental Biology, University of Colorado, Boulder, Colorado (147)

GORDON E. STONE, Department of Anatomy, University of Colorado School of Medicine, Denver, Colorado (161)

ELTON STUBBLEFIELD, Section of Cell Biology, Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas (25)

R. A. TOBEY, Biomedical Research Group, Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico (347)

JACK VAN'T HOF, Biology Department, Brookhaven National Laboratory, Upton, New York (95)

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[6] *Present address:* Department of Biology, Faculty of Education, Shimane University, Matsue, Shimane-Ken, Japan

PREFACE

Volume III of this treatise continues to present techniques and methods in cell research that have not been published or have been published in sources that are not readily available. Much of the information on experimental techniques in modern cell biology is scattered in a fragmentary fashion throughout the research literature. In addition, the general practice of condensing to the most abbreviated form materials and methods sections of journal articles has led to descriptions that are frequently inadequate guides to techniques. The aim of this volume is to bring together into one compilation complete and detailed treatment of a number of widely useful techniques which have not been published in full detail elsewhere in the literature.

In the absence of firsthand personal instruction, researchers are often reluctant to adopt new techniques. This hesitancy probably stems chiefly from the fact that descriptions in the literature do not contain sufficient detail concerning methodology; in addition, the information given may not be sufficient to estimate the difficulties or practicality of the technique or to judge whether the method can actually provide a suitable solution to the problem under consideration. The presentations in this volume are designed to overcome these drawbacks. They are comprehensive to the extent that they may serve not only as a practical introduction to experimental procedures but also to provide, to some extent, an evaluation of the limitations, potentialities, and current applications of the methods. Only those theoretical considerations needed for proper use of the method are included.

Finally, special emphasis has been placed on inclusion of much reference material in order to guide readers to early and current pertinent literature.

DAVID M. PRESCOTT

September, 1968

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Chapter 1

Measurement of Cell Volumes by Electric Sensing Zone Instruments

R. J. HARVEY

Department of Biology, State University of New York at Buffalo, Buffalo, New York

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I. Introduction

The measurement of particle concentrations and volumes by electric sensing zone instruments has found wide application in recent years. Such instruments are capable of rapid and very accurate measurement of particle concentrations, and of more or less accurate determinations of volume distributions of particle populations.

The original biological application of the technique was in routine blood cell counting (Brecher *et al.*, 1956). Kubitschek (1958, 1960) introduced modifications which permitted counting of bacterial cells, and

pointed out that electric sensing zone instruments were capable of measuring cell volume distributions as well as numbers. With instruments currently available, particles ranging in volume from about 0.1 to 5×10^5 μ^3 (for corresponding spherical particles, a range of diameters from 0.6 to 100 μ) can be counted and measured, provided only that the particles can be suspended in a medium of suitable electrical conductivity. This size range includes a wide variety of types of cells, including bacteria, the smaller protozoa, red and white blood cells, and tissue culture cells, all of which have been successfully studied by this method.

A recent review by Kubitschek (1967) provides a comprehensive description of the methodology and the underlying theory of the electric sensing zone technique. In an earlier review Berg (1965) describes electric, optical, and sonic sensing zones for particle counting and measurement.

The present paper is concerned with the accuracy of measurement of volume distributions by electric sensing zone instruments now in use. These instruments are: (1) The Coulter Counter Model B (Coulter, 1953, Coulter Electronics Inc., Hialeah, Florida); (2) The instrument described by Harvey and Marr (1966); (3) The Celloscope 101 (Particle Data Inc., Elmhurst, Illinois); (4) The Nuclear Chicago particle measurement system (Nuclear Chicago Corp., Des Plaines, Illinois). There is little question of the accuracy of all these instruments in the counting of cells. The accuracy and fidelity of measurement of volume is by no means as certain nor is it easily determined. Faced with this uncertainty most workers have evidently been content to accept the apparent volume distributions measured by their instrument as accurately representing the real distribution. More rigor is shown by Kubitschek (1967) who applied the criterion that measurements should be made under conditions where the variance of a standard suspension of particles is at a minimum.

The only attempt at rigorous evaluation of the fidelity of measurement of volume distribution is that of Harvey and Marr (1966). The volume distribution of a suspension of latex spheres was measured by electron microscopy. The electron microscopic measurements were taken as a primary standard, and statistical comparisons of distributions measured by the electric sensing zone instrument with the primary standard were used as the criteria for fidelity and accuracy of measurement. Their results are recapitulated in this paper, and the technique is used to evaluate the performance of instruments not tested in their original study.

The value of electron microscopic measurements as a primary standard is not completely established. Bonse and Hart (1966) have shown that the mean diameters of latex spheres measured by electron microscopy agree

very well with values calculated from X-ray diffraction measurements. Smaller particles (diameters $< 0.5\ \mu$) tended to be measured as too large, and larger particles (diameters $> 2\ \mu$) were measured as too small by electron microscopy, but in the range of diameters 0.5–2 μ the agreement was within 1%. Since the X-ray diffraction data were shown to be free of any detectable systematic errors these results provide reasonable evidence of the validity of electron microscopic data as a primary standard.

II. Design of Equipment

A. The Electric Sensing Zone Transducer

The transducer is a cylindrical aperture separating two electrode chambers filled with electrolyte. The aperture is commonly fabricated from ruby or glass, and may have a diameter of 10 μ to 1 mm, the length usually being about equal to the diameter.

The particles being measured are suspended in the outer electrode chamber and pumped through the aperture. When a dc voltage is applied between the electrodes, the aperture and a small hemispherical volume at either end of it provide the major resistance to flow of current and form the sensing zone of the transducer. During the passage of a cylindrical particle of volume v through the aperture, the resistance R of the sensing zone will be increased. The magnitude, ΔR of the resistance increase is a function of the particle volume (Kubitschek, 1958);

$$\frac{\Delta R}{R} = \frac{v}{V}\left(\frac{\rho}{\rho - \rho_0} - \frac{a}{A}\right)^{-1} \tag{1}$$

where V = the volume of the sensing zone
A = area of the aperture normal to its axis
ρ_0 = resistivity of the electrolyte
ρ = resistivity of the particle
a = cross-sectional area of the particle normal to the axis of the aperture

For particles with $a \ll A$ Eq. (1) reduces to:

$$\frac{\Delta R}{R} \simeq \frac{v}{V} \cdot \frac{\rho - \rho_0}{\rho} \tag{2}$$

If the resistivity of the particle is much greater than the resistivity of the electrolyte, the response will become independent of the resistivity of the particle:

$$\frac{\Delta R}{R} \simeq \frac{v}{V} \tag{3}$$

Thus for particles with diameters much smaller than that of the aperture the resistance change produced by the particle is proportional to its volume. When the diameter of the particle is 10% of the aperture diameter ($a/A = 0.01$) the deviation from linearity will be 1%, and this can be taken as setting a practical upper limit for the particle size which can be measured with a given aperture.

This analysis holds for particles in the shape of a right cylinder. Under some circumstances particles of different shapes but equal volumes can give rise to different resistance changes. Gregg and Steidley (1965) showed that the resistance change produced by a disk with its face normal to the axis of the aperture was three times the resistance change produced by a sphere of equal volume. This is due to the fact that current flow lines will not follow flat surfaces normal to the flow of current, as in the case of the disk. Thus a volume of high effective resistivity is established greater than the volume of the disk. This will not be the case for particles which have no sharp edges or flat faces, and have smooth surfaces, a description which will fit most biological cells. For such particles current flow lines will closely follow the surface, and the resistance change will not be greatly influenced by particle shape. Kubitschek (1967) has calculated that, when compared to a sphere of the same volume, a prolate spheroid with an aspect ratio of 4:1 will give rise to a resistance change only 3% greater.

The increase in resistance produced by the passage of a particle results in a transient change in the voltage drop across the sensing zone, proportional to the resistance change, and this voltage pulse constitutes the output of the transducer. The form of the pulse would ideally be a square wave, but the actual pulse will not have this shape, primarily because of the gradient of the electrical field and the capacitance of the aperture. From the measurements of Gregg and Steidley (1965) this capacitance is of the order of 100 picofarads, giving the aperture a time constant of 1–5 μsec, depending upon its resistance. Hence the minimum rise time of the pulse would be 2–10 μsec. The capacitance of the particle could also affect the rise time. However, Gregg and Steidley (1965) have shown that for typical mammalian cells the time constant due to this capacitance is about 0.4 μsec, making this effect relatively unimportant.

After the rise, the pulses may be flat-topped, but a slight concavity is often observed (Kubitschek, 1967), which can possibly be attributed to the capacitances of the particle and the aperture. The pulse has a finite decay time, due again to the field gradient and aperture capacitance. Under normal conditions of operation, the mean transit time of particles through the sensing zone will be 10–30 μsec, depending on the dimensions of the aperture and the pressure applied across it. The overall pulse duration will be of this order of magnitude.

The transducer described above is used in all instruments with only minor modifications. The pulses produced are amplified and measured and it is in the amplification and measurement systems that the instruments differ.

B. Pulse Amplification and Measurement

1. Coulter Counter Model B

This instrument employs simple amplification of pulses by a vacuum tube amplifier. The time constant of the amplifier is about 30 μsec, which results in about a 10-fold increase in the duration of pulses during amplification. The amplified pulses are measured by a single-channel analyzer, which allows manual measurement of size distributions.

2. Instrument Described by Harvey and Marr (1966)

The pulses from the transducer are first amplified by a Tennelec 100B preamplifier (Tennelec Instrument Co., Inc., Oak Ridge, Tennessee). The pulses have a duration of 200 to 300 μsec at the output of the preamplifier, which is about 10 times greater than that expected from the mean transit time of particles through the sensing zone used. Since the preamplifier has a rise time of less than 1 μsec, this distortion of the pulses must result from the design of the constant current device used to provide the voltage between the electrodes. The pulse shapes are restored by differentiation and integration using a Tennelec TC200 linear amplifier (Tennelec Instrument Co., Inc.). This procedure reduces pulse widths to about 10 μsec, and the amplitude of the resulting pulse is proportional to the true amplitude of the input pulse above any arbitrary baseline. As a result the true amplitudes of coincident pulses can be measured. A final differentiation produces pulses of suitable form for acceptance by a Nuclear Data 180-FM 512-channel pulse-height analyzer (Nuclear Data, Inc., Palatine, Illinois). The analyzer measures each pulse and stores it as a count in a channel of the memory unit, the channel number being proportional to the amplitude of the pulse.