LA-3132-MS

LOS ALAMOS SCIENTIFIC LABORATORY
OF THE UNIVERSITY OF CALIFORNIA o LOS ALAMOS NEW MEXICO

BIOLOGICAL AND MEDICAL RESEARCH GROUP (H-4) OF THE HEALTH DIVISION -- ANNUAL REPORT JULY 1963 THROUGH JUNE 1964

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BIOLOGICAL AND MEDICAL RESEARCH GROUP (H-4) OF THE HEALTH DIVISION -- ANNUAL REPORT JULY 1963 THROUGH JUNE 1964

Group Leader, W. H. Langham
Division Leader, T. L. Shipman

Contract W-7405-ENG. 36 with the U. S. Atomic Energy Commission

CHAPTER 1

INTRODUCTION

(a) Program Orientation

During this report period (FY 1964), considerable expansion of molecular and cellular level studies occurred with the arrival of new personnel. Projects involving clinical applications of radiation and radioactive isotopes have been brought to a reasonable conclusion, and the Clinical Investigations Section has been discontinued as a result of the termination of Dr. C. C. Lushbaugh. An increasing amount of the effort of the Low-Level Counting Section has been devoted to improving electronic cell counting techniques and developing suitable instrumentation for other sections. These increasing applications of physics to biological problems are responsible for changing the name of the section to Biophysics.

During FY 1965, the categories of environmental radiation studies and health physics will be further curtailed and the effort directed toward biophysics and instrumentation in support of the fundamental molecular and cellular level studies. During FY 1965-1966, studies of the toxicology of radionuclides will be continued and perhaps somewhat increased. Studies of the genetic effects of radiation will continue at a decreasing level, in view of the results obtained thus far, and the effort transferred to studies on somatic effects of radiation in the sublethal dose range using primates.

Construction of a new occupational health laboratory in FY 1965 for occupancy by the Industrial Hygiene Group (H-5) and the Field Studies Group (H-8) will free considerable

office and laboratory space in the Health Research Laboratory to accommodate anticipated expansion of the biological and medical research staff.

FY 1965 research activities will be devoted principally to the following program categories at approximately the percentage levels indicated:

06-01 Somatic Effects of Radiation (17 per cent)

06-02 Radiation Genetics (18 per cent)

06-04 Molecular and Cellular Level Studies (62 per cent)

In addition, other projects will continue in environmental radiation studies (06-05), radiological and health physics and instrumentation (06-06), and selected beneficial applications (06-10).

(b) Terminations

The following terminations occurred during the present report period:

P. A. Goldman, research assistant, Molecular Radiobiology Section.

N. C. Brown, technician, Mammalian Radiobiology Section.

(c) Leave of Absence

Dr. Irene U. Boone, staff member, Cellular Radiobiology Section, has been granted an additional year's leave of absence to practice medicine in the Los Alamos Medical Center.

(d) New Hires

The recruiting campaign of the past two years to fill positions created in expanding the molecular and cellular level studies has resulted in the addition of a number of extremely well qualified personnel. New hires during FY 1964 are as follows:

GROUP H-4

BIOMEDICAL RESEARCH

W. H. Langham, Ph.D., Leader
D. G. Ott, Ph.D., Alt. Group Leader
O. S. Johnson, B.S., Asst. Ldr. for Administration
E. M. Sullivan, Group Secretary

MAMMALIAN METABOLISM

C. R. Richmond, Ph.D., Leader

Staff Member

J. E. Furchner, Ph.D.

Research Assistants

G. A. Drake, B.S.
J. S. Findlay, B.S.
J. E. London, B.S.

Technical Staff

S. Cordova
R. Martinez

*Casual employee.

MAMMALIAN RADIOBIOLOGY

J. F. Spalding, Ph.D., Leader

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O. S. Johnson, B.S.

Research Assistants

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G. E. Oakley, B.A.

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R. F. Archuleta
J. E. Atencio
L. Ortiz
A. Trujillo
D. G. Valdez
F. Valdez
E. A. Vigil

BIOPHYSICS

M. A. Van Dilla, Ph.D., Leader

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M. J. Fulwyler, B.S.
J. H. Larkins, B.S.*
J. D. Perrings

Technical Staff

R. L. DePriest, B.S.

Electronics Technicians

L. J. Carr
A. R. Vigil

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Staff Members

E. C. Anderson, Ph.D.
I. U. Boone, M.D.**
E. A. Hyatt, Ph.D.
A. G. Saponara, Ph.D.
R. A. Tobey, Ph.D.

Research Assistants

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S. Carpenter, B.A.
S. H. Cox, B.A.
P. M. LaBauve, B.A.
P. C. Sanders, M.S.
F. Sapir, M.S.

Technical Staff

N. J. Basmann
V. M. Gibbs

MOLECULAR RADIOBIOLOGY

F. N. Hayes, Ph.D., Leader

Staff Members

C. T. Gregg, Ph.D.
D. E. Hoard, Ph.D.
V. N. Kerr, M.A.
A. Murray, M.S.
R. L. Ratliff, Ph.D.
G. R. Shepherd, Ph.D.
D. A. Smith, Ph.D.
T. T. Trujillo, B.S.
D. L. Williams, M.S.

Research Assistants

G. T. Fritz, B.S.
E. Hansbury, M.A.
R. E. Hine, B.S.
E. H. Lilly, B.S.
B. J. Noland, B.A.
C. N. Roberts, B.A.

Technical Staff

V. E. Mitchell

**Leave of absence.

ELECTRONIC CELL SIZING. I. EFFECT OF APERTURE CURRENT

(M. A. Van Dilla, N. J. Basmann, and M. J. Fulwyler)

INTRODUCTION

A method of measuring accurately and rapidly the volume distribution of erythrocytes and other cells would have broad applications in biological research, medical research, and clinical diagnosis. At this Laboratory, there is currently a need for such a device in studies of (a) cell synchronization, and (b) the effects of radiation on the hematopoietic system. Work originated by Lushbaugh (1) on red blood cell sizing with a Coulter counter has been continued with some improvement in experimental apparatus and technique, our objective being to obtain true volume distributions with minimum instrumental distortion. We report here the influence of aperture current on the measured distribution function and show that large aperture currents distort the shape of the distribution and substantially decrease the apparent volume. For this reason, we recommend that the aperture current used be the smallest possible.

METHODS AND RESULTS

Lushbaugh et al. (1) have reported that increasing aperture current improves the resolution of red blood cell volume distributions obtained with a Coulter counter and that the resulting bimodal distribution is due to the existence of two volumetrically distinct subpopulations. We decided to investigate this idea for the following reasons: (a) this finding is controversial (2); (b) there is no theoretical basis for resolution improvement with increased aperture current; and (c) it seems reasonable to minimize aperture current in order to minimize possible electrical (or other) effects on the cells and physical phenomena at the aperture.

Following Lushbaugh et al. (1), the diluent used to suspend particles and cells was 0.85 per cent saline stabilized at pH 6.7 with a phosphate buffer. The two apertures described in Table 1 were used. Aperture currents used were 50 μA and 2000 μA, the higher value being close to that used by Lushbaugh to obtain a distinctly bimodal distribution. Data on the red cells of 1 adult (1A) are presented.

TABLE 1. APERTURE CONDITIONS USED TO INVESTIGATE EFFECT OF APERTURE CURRENT

Designation	Diameter (μ)	Length (μ)	Average Transit Time at 12 to 14 in. Mercury (μsec)	Pulse Rise Time (μsec)
70/56	70	56	8	~8
75/400	75	400	78	~10

The difference in pressure across the aperture was maintained at 12 to 14 in. mercury, resulting in the average transit times listed in Table 1. Pulses generated by the traversal of a cell through the aperture were amplified by a LASL Model 101 amplifier (integrating time 6 μsec, differentiating time 65 μsec) and sorted by a Model 34-12 RIDL 400-channel pulse height analyzer.

Pulse height spectra at both high and low current in both apertures are shown in Figs. 1, 2, 3, and 4. Note that all have different shapes. The short aperture gives bimodal distributions at both currents, with the valley between the two peaks deeper at the larger current. The longer aperture gives a broad asymmetric distribution at 50 μA and a distinct bimodal distribution at 2000 μA. Calibration of pulse height in terms of apparent volume was performed by measuring a saline suspension of ragweed pollen (diameter about 19 μ, volume about 3700 μ^3) under similar conditions; we have found experimentally that pollen grains are unaffected by the currents used. On this basis, apparent volumes were calculated and are listed in Table 2.

DISCUSSION

It is clear that all of the "volume" distributions shown in Figs. 1, 2, 3, and 4 cannot be correct and indeed may all be incorrect -- which is probable (see paper II in this series).

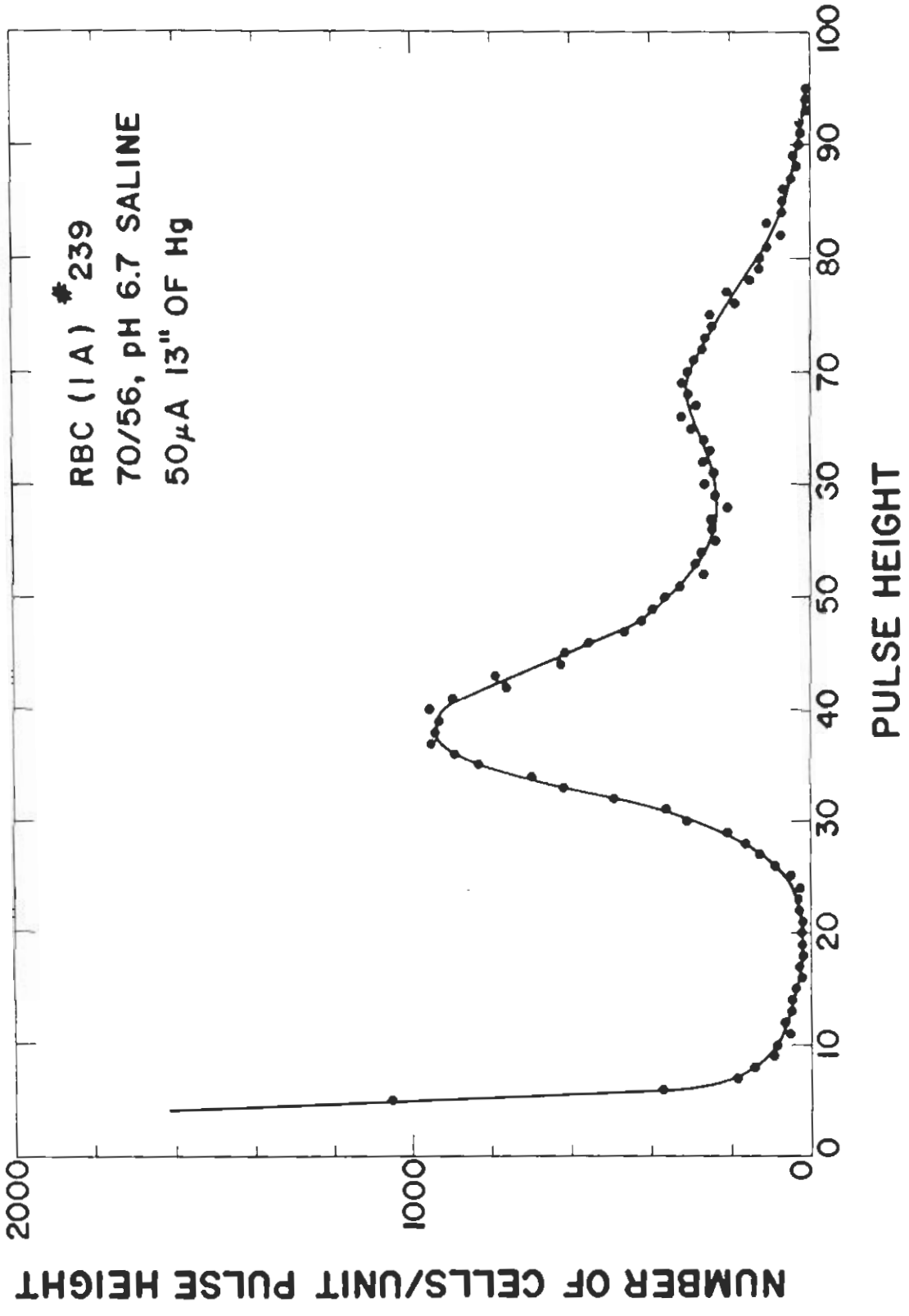

Fig. 1. Distribution of apparent volume of red blood cells of an adult female (1A) using pH 6.7 isotonic saline in 70/56 aperture, I_a = 50 μA.

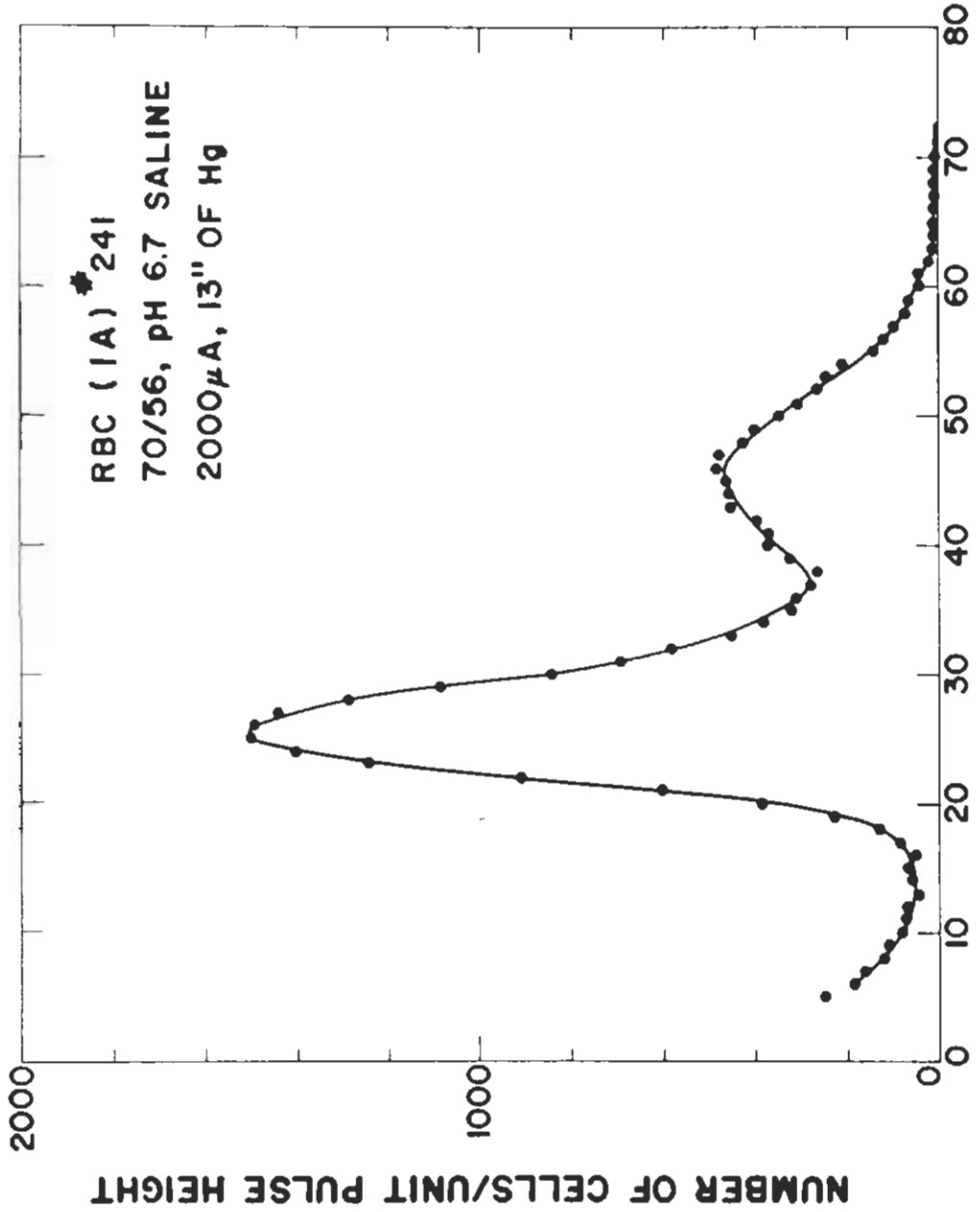

Fig. 2. Distribution of apparent volume of red blood cells of an adult female (1A) using pH 6.7 isotonic saline in 70/56 aperture, I_a = 2000 μA.

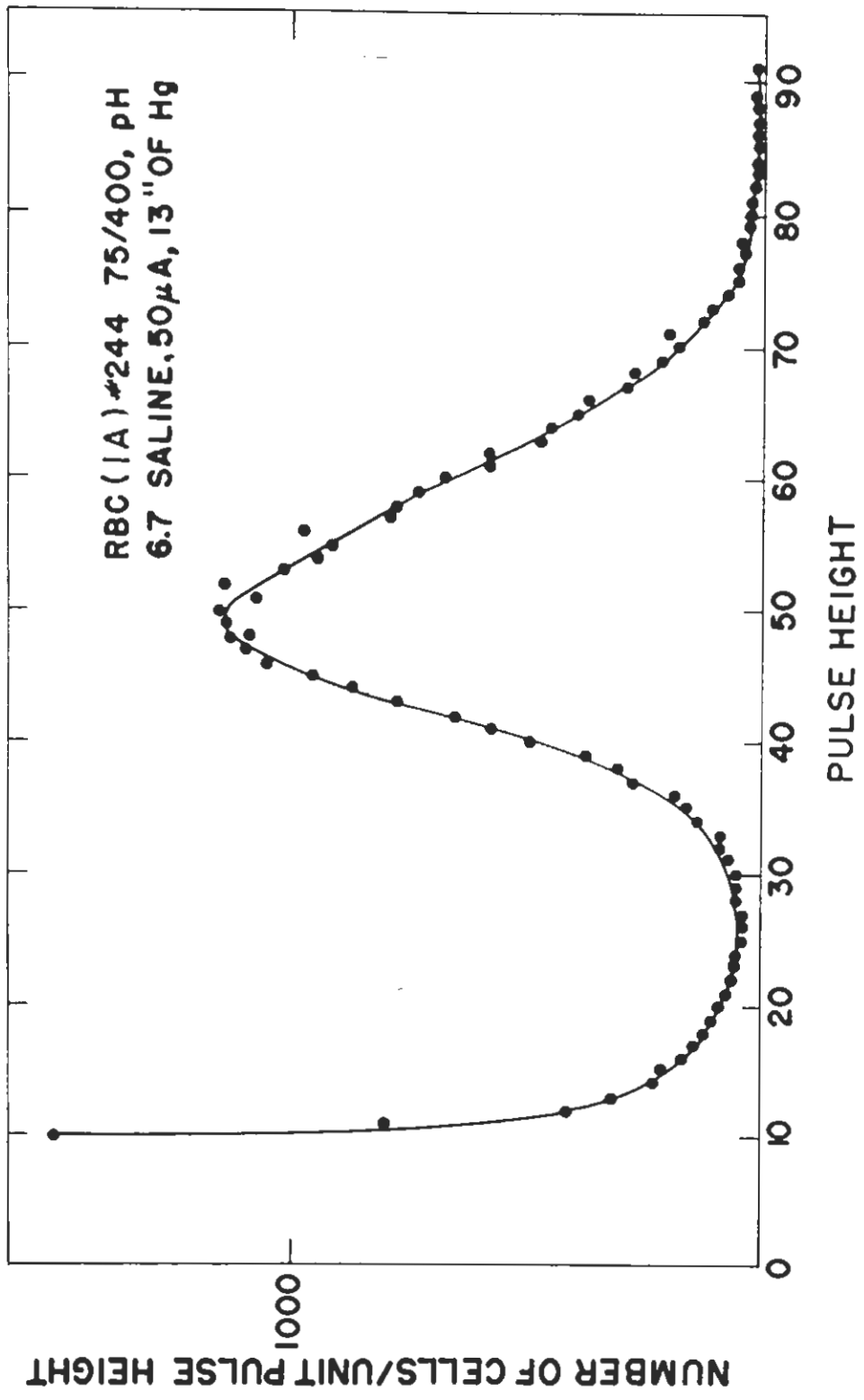

Fig. 3. Distribution of apparent volume of red blood cells of an adult female (1A) using pH 6.7 isotonic saline in 75/400 aperture, I_a = 50 μA.

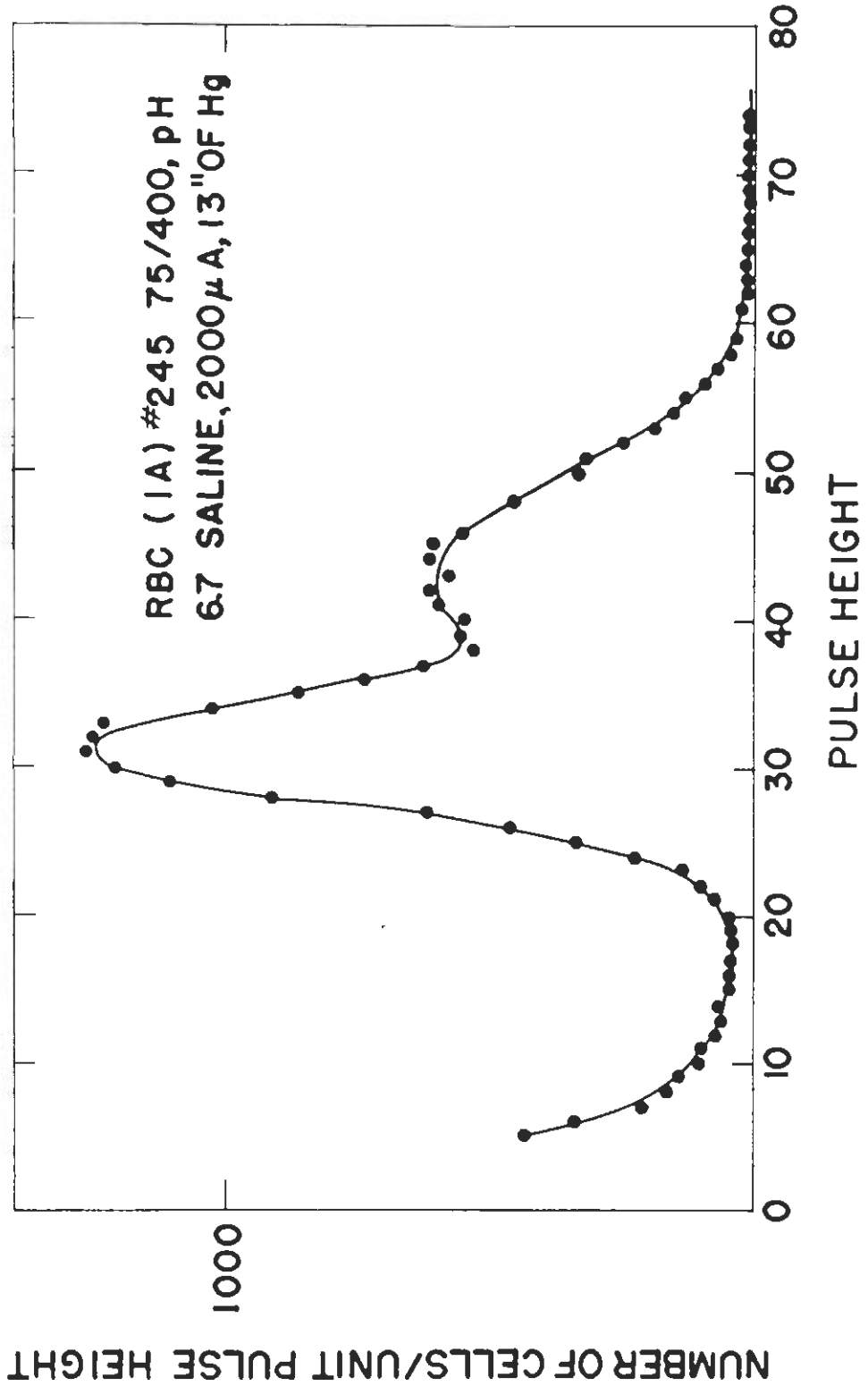

Fig. 4. Distribution of apparent volume of red blood cells of an adult female (1A) using pH 6.7 isotonic saline in 75/400 aperture, I_a = 2000 μA.

TABLE 2. MODAL AND MEAN RED CELL "VOLUME" FOR AN ADULT (1A); RAGWEED POLLEN CALIBRATION

Aperture	Apparent Volume (μ^3)	I_a = 50 μA	I_a = 2000 μA
70/56	Mean	108	81
	First mode	84	61
	Second mode	148	112
75/400	Mean	114	85
	First mode	107	72
	Second mode	not present	100

The effect of large aperture current is certainly real; it not only changes the spectral shape but also decreases the mean "volume" by a factor of 0.75. The reasons for these effects are unknown. We can probably rule out electrical heating of aperture content, since calculation of the average temperature rise for 2000 μA results in a 0.7° C rise for the 70/56 and a 3° C rise in the 75/400 apertures. The best guess at the present writing is some sort of electrical effect on the red cell. Professor A. C. Burton (Department of Biophysics, Faculty of Medicine, University of Western Ontario) has preliminary data on behavior of red cells in saline when microelectrodes carrying current are brought close; the cells (normally biconcave discs) near one electrode crenate, and those near the other become sickle-shaped. Reversing the polarity reverses these shape changes; turning off the current restores the original disc shape. This seems a promising lead to investigate.

All else being constant, ragweed pollen spectra, taken at aperture currents between 50 and 2000 μA, show no change in

spectral shape; the proportionality between modal pulse height and aperture current is as expected. Hence, this pollen is an inert, constant calibration source. The pollen volume, taken as 3700 μ^3, is based on microscopic measurement of diameter, which is uncertain to about 10 per cent because the pollen grains, although nearly spherical, have a rough knobby surface. Thus, the absolute volume calibration could be off by as much as 30 per cent.

It should be emphasized that even at low current the spectra (Figs. 1 and 3) are quite different. We have reason to believe that the short aperture introduces serious distortion and that the long aperture is better but not ideal. Also, pH 6.7 saline may not be the best diluent, since microscopic examination shows that it results in crenated red cells. We will discuss this in succeeding papers.

Finally, we wish to point out that the enhanced bimodal spectrum generated at high aperture currents may or may not be more useful in practical application than the true volume distribution. It does seem, however, erroneous to state that red cell spectra generated in short apertures by high currents represent two volumetrically distinct subpopulations.

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ELECTRONIC CELL SIZING. II. INFLUENCE OF PHYSICAL PHENOMENA ON MEASURED SPECTRA

(M. A. Van Dilla, N. J. Basmann, and M. J. Fulwyler)

INTRODUCTION

The importance of a fast, reliable, and accurate method of measuring the volume distribution of cells suspended in an appropriate liquid medium has been pointed out in part I of this series. Here we present results obtained using very uniform polystyrene spheres and much larger, less uniform ragweed pollen grains. It is shown that improper choice of aperture conditions yields a measured distribution very different from the known volume distribution. Thus broad, asymmetric, and bimodal distributions are generated by polystyrene spheres of almost identical volume. With proper aperture conditions, the same spheres generate spectra very close to the correct volume distribution.

METHODS

The experimental arrangement was described in part I of this series with the exception that two apertures of smaller diameter were investigated (Table 1). The polystyrene spheres (Dow Chemical Company, courtesy of Dr. John Vanderhof) had a nominal mean diameter of 3.04 μ; electron microscopy showed the actual mean diameter to be 2.81 μ. Our best estimate of the standard deviation of the diameter distribution is about 0.5 per cent. Thus, we have a particle distribution of accurately known parameters and of remarkably uniform size. The ragweed pollen cannot be so accurately characterized. The grains are almost spherical but with a somewhat knobby surface, making microscopic determination of volume uncertain. Our best estimate of effective diameter is 19 μ, with an uncertainty of 1 to 2 μ. The diameter distribution has a standard deviation of about 5 per cent.

RESULTS AND DISCUSSION

The spectra generated by the very uniform polystyrene spheres passing through the 70/56 aperture are shown in Fig. 1, and it is clear that this is certainly not the correct volume

TABLE 1. APERTURE CONDITIONS USED TO INVESTIGATE PHYSICAL PHENOMENA

Designation	Diameter (μ)	Length (μ)	Average Transit Time at 12 to 14 in. Mercury (μsec)	Pulse Rise Time (μsec)
70/56	70	56	8	~8
75/400	75	400	78	~10
30/59	30	59	10-15	~8
30/225	30	225	50	20

distribution. At the usual pressure differential (12 to 14 in. mercury), the spectrum is very broad, asymmetric, and bimodal. At a lower pressure differential (2 in. mercury) and thus a longer transit time, the shape changes somewhat and the mean pulse height increases. It seems, then, that an important consideration is the ratio of average transit time to the pulse rise time, the latter quantity being determined by the amplifier-integrating time and the RC time constant at the amplifier input. If the average particle is not in the aperture long enough for amplifier output pulse to rise to its maximum value, then the slower particles will generate larger pulses than the faster ones -- even if they all have identical volume. Oscilloscopic examination of the amplifier output supports this. In addition to this timing effect is the unknown and perhaps quite nonuniform electrical field in such a thin aperture. The fluid flow pattern and its influence on particle trajectory through the aperture may also play an important role. In any event, the measured volume distribution of these polystyrene spheres is in error; the reasons are poorly understood.

Ragweed pollen, measured under the same conditions, gives much better results. The spectra are roughly symmetrical with a resolution of 30 per cent. This is about what one

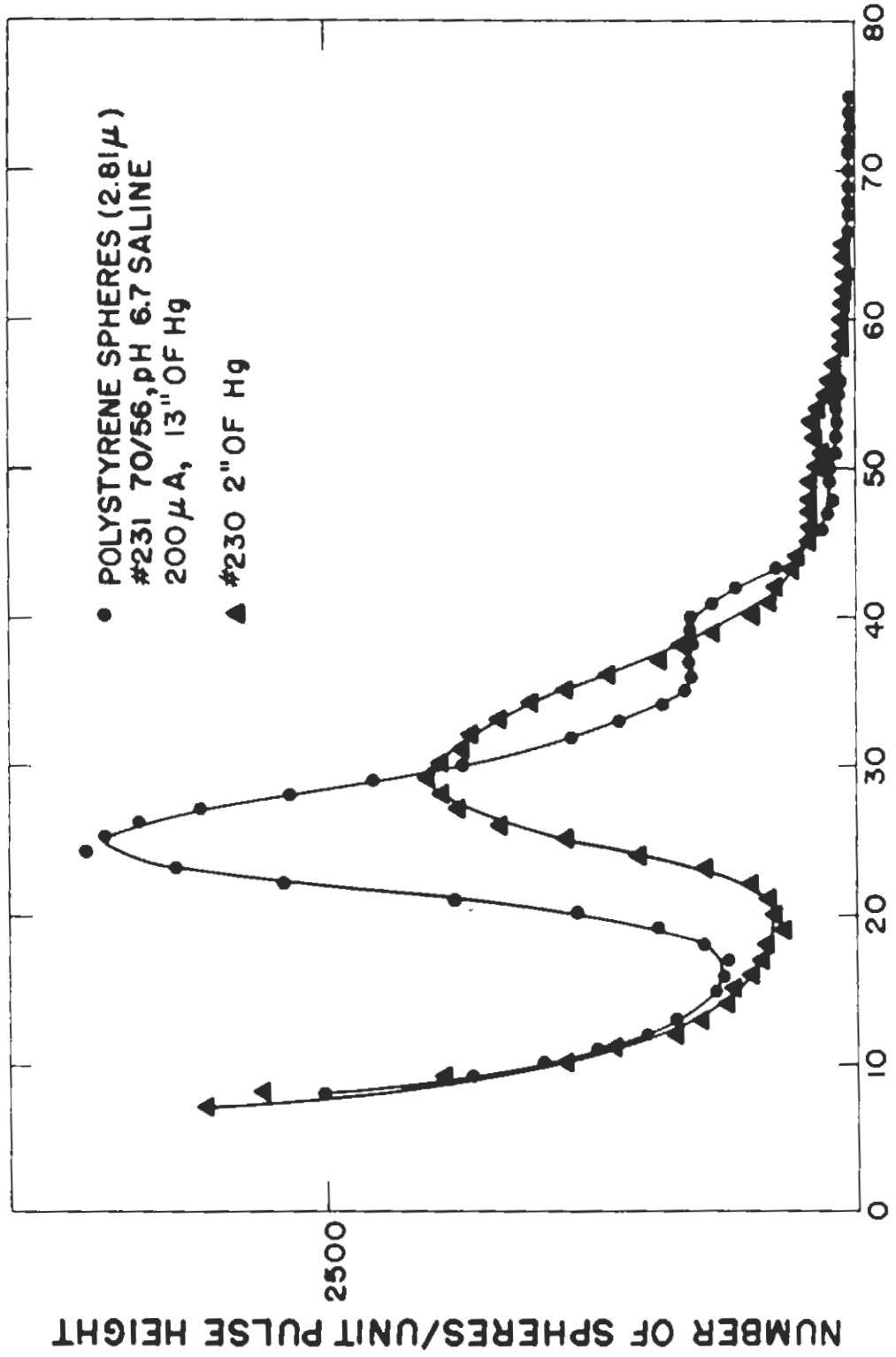

Fig. 1. Distribution of apparent volume of polystyrene spheres (diameter = 2.81 μ) suspended in pH 6.7 isotonic saline in 70/56 aperture.

would expect from the microscopic results. The mean pulse height increases 13 per cent at the lower pressure differential (2 in. mercury), showing the same timing effect noticed with the polystyrene spheres. Thus, the broader pollen distribution generates a narrower spectrum than the very uniform polystyrene spheres, a startling reversal of expectation! Although the explanation is not at all clear, two possibilities come to mind. The first is concerned with the probable nonuniform electrical field in a short aperture; a large particle will tend to average out nonuniformities, while a small particle will not. Second, the much larger and heavier pollen grains may traverse the aperture on a more uniform trajectory than the polystyrene spheres, in which case spectral broadening due to the timing effect and field nonuniformities will be minimal. In spite of our inadequate understanding of the physical phenomena involved, we conclude that the short 70/56 aperture produces very poor results for 3-μ polystyrene spheres and reasonably good results for 19-μ pollen grains, making one suspicious of results with 8-μ red blood cells.

With the long 75/400 aperture, the polystyrene spheres produce a better, more symmetrical, and narrower spectrum with resolution improved to 15 per cent. The ragweed pollen spectrum is also improved; resolution is now 25 per cent. Thus, making average transit time long compared to pulse rise time (ratio $\approx$ 8) by lengthening the aperture has given a better result. But still the polystyrene sphere spectrum is much broader than it should be. Perhaps part of this is due to noise, and the signal/noise ratio can be greatly improved in apertures of smaller diameter. This was the motivation for the following experiments with 30-μ apertures.

With a short 30/59 aperture, polystyrene spheres generate the spectrum shown in Fig. 2, which is again highly distorted and even more distinctly bimodal. Although the signal/noise ratio is now much better, it is obvious that the spectrum is not. With a long 30/225 aperture, the greatly improved spectrum of Fig. 3 is obtained. Now the sphere peak is very sharp (resolution = 6.5 per cent). Indeed, most of its width is due to noise broadening of 2.1 channels, as shown by the pulser peak. If broadening effects propagate as do errors in summation (1,2), then noise effects can be removed, leaving a residual broadness or resolution of 3.8 per cent. This result is now close to the actual volume distribution of the spheres and indicates that we now have a good method for measuring the true volume distribution of these particles.

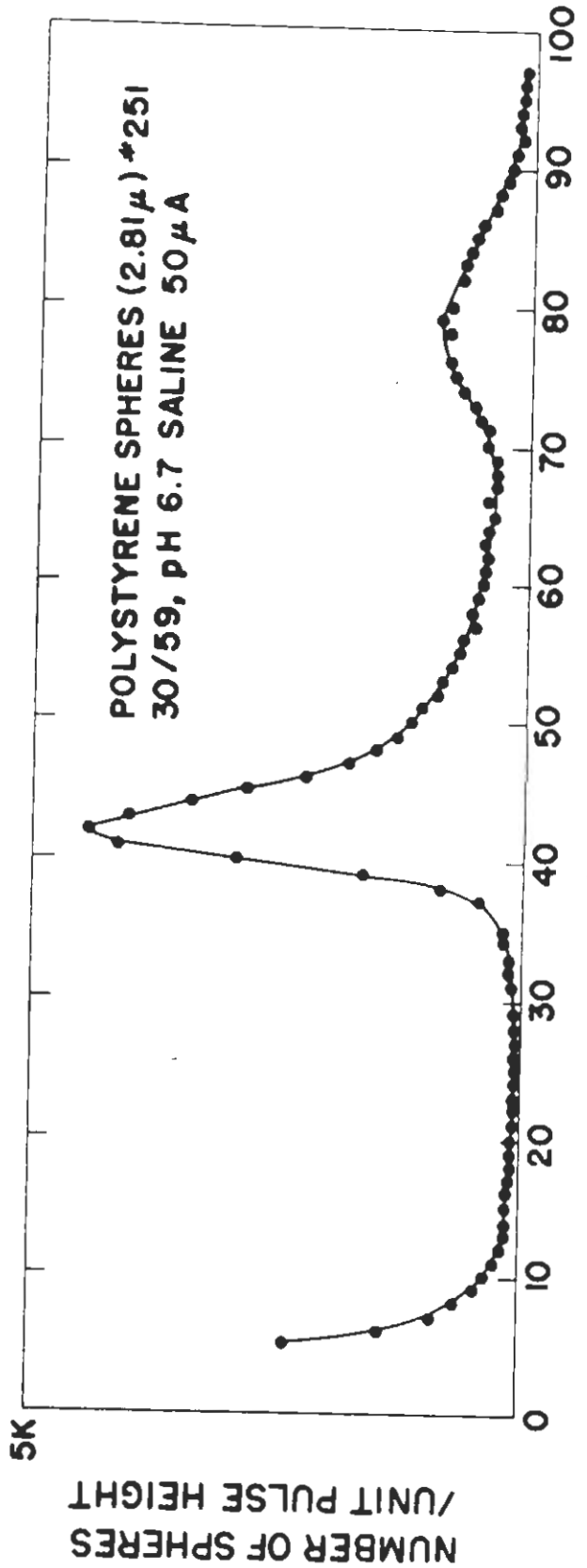

Fig. 2. Distribution of apparent volume of polystyrene spheres (diameter = 2.81 μ) suspended in pH 6.7 isotonic saline in 30/59 aperture.

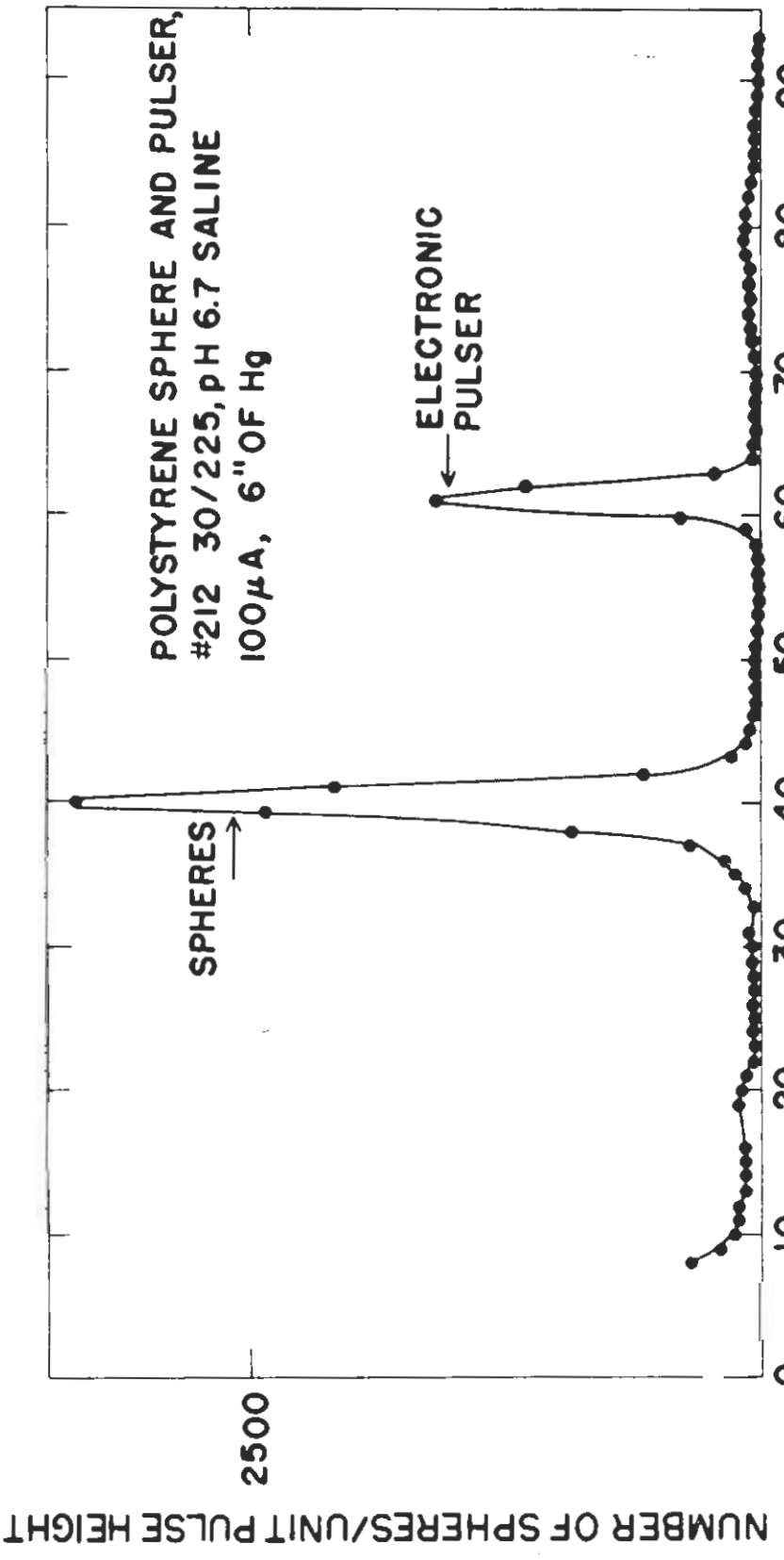

Fig. 3. Volume distribution of polystyrene spheres (diameter = 2.81 μ) suspended in pH 6.7 isotonic saline in 30/225 aperture.

Oscillographic examination of the amplifier output pulse shape shows that timing is good (i.e., all pulses rise to maximum value before the particles emerge from the aperture); this is good electronic evidence for proper operation. The polystyrene spheres have a tendency to form aggregates; we have observed smaller peaks at 2, 3, and even 4 times the main peak pulse height due to the presence of 2, 3, or 4 spheres in the aperture at one time. These multiplet peaks can be almost entirely eliminated by filtration of the suspension through a 10-μ membrane filter. These observations, which are similar to those of Kubitschek (3), are further evidence of proper operation.

We summarize these results in the following way:

(a) There are anomalies due to purely physical effects such as electrical field distribution, hydrodynamics, or particle trajectory that can badly distort the measured spectrum.

(b) "Large" particles (i.e., ratio of particle diameter to aperture diameter = 0.26) give good results in short or long apertures.

(c) "Small" particles (i.e., ratio of particle diameter to aperture diameter = 0.04 to 0.09) give good results only in the long 30/225 aperture.

(d) Red blood cells will probably give the best results in the 30/225 aperture.

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(2) P. R. J. Burch, D. Hughes, T. A. Sinuma, T. R. Overton, and D. B. Appleby, In: Whole Body Counting, International Atomic Energy Agency, Vienna (1962), pp. 59-69.

(3) H. E. Kubitschek, Research 13, 128 (1960).

ELECTRONIC CELL SIZING. III. ERYTHROCYTES

(M. A. Van Dilla, N. J. Basmann, and M. J. Fulwyler)

INTRODUCTION

The importance and current applications of this research have been pointed out in the previous papers of this series, in which we also discussed several obstacles in the way of good volume measurement. Reported here are results of measurements of red blood cells with the best system we have developed to date. The observed distribution for human erythrocytes is unimodal, approximately symmetrical with a slight skew to the high side, and with a width (resolution) between 30 and 40 per cent. Internal evidence, in addition to supporting data from other workers, leads us to believe that these results are close to the true volume distribution.

METHODS AND RESULTS

The same experimental equipment and techniques described in parts I and II of this series were used. Results on 5 adults using pH 6.7 isotonic saline as the diluent are listed in Table 1. In addition to the usual minimal aperture current (50 μA), a high value (800 μA) was used to check the current effect described in part I of this series. A typical spectrum is shown in Fig. 1. Calibration was accomplished with polystyrene spheres (2.81 μ measured diameter; see paper II); we assume they are unaffected by the aperture currents used. The modal value, V, is probably close to the true modal volume at 50 μA but certainly not at 800 μA (which reduces V by about 40 per cent). This effect is similar to that found with the larger apertures (see part I), but now bimodal shapes are not observed. An increased degree of skew, as indicated by the asymmetry factor b/a, is also observed.

DISCUSSION

As observed in paper I of this series, high aperture current affects the measured spectrum by substantially reducing apparent volume; the shape change is small, however, and consists only of a small increase in asymmetry. At both currents, the shape is unimodal.

TABLE 1. HUMAN ERYTHROCYTE MEASUREMENTS IN A 30/225 APERTURE (pH 6.7 saline, 12- to 14-in. mercury)

Subject	I_a = 50 μA			I_a = 800 μA			$\frac{V_{800}}{V_{50}}$
	V[a]	Resolution (per cent)	b/a[b]	V[a]	Resolution (per cent)	b/a[b]	
1A	81	33	1.03	46	35	1.46	0.57
2A	76	33	1.05	43	32	1.33	0.57
3A	74	34	1.13	43	37	1.40	0.58
4A	78	35	1.17	--	--	--	--
5A[c]	55	44	1.10	34	40	1.53	0.61

[a]V is modal "volume" relative to polystyrene spheres (see text).

[b]b/a is a measure of spectrum asymmetry (see text).

[c]Known microcytic case.

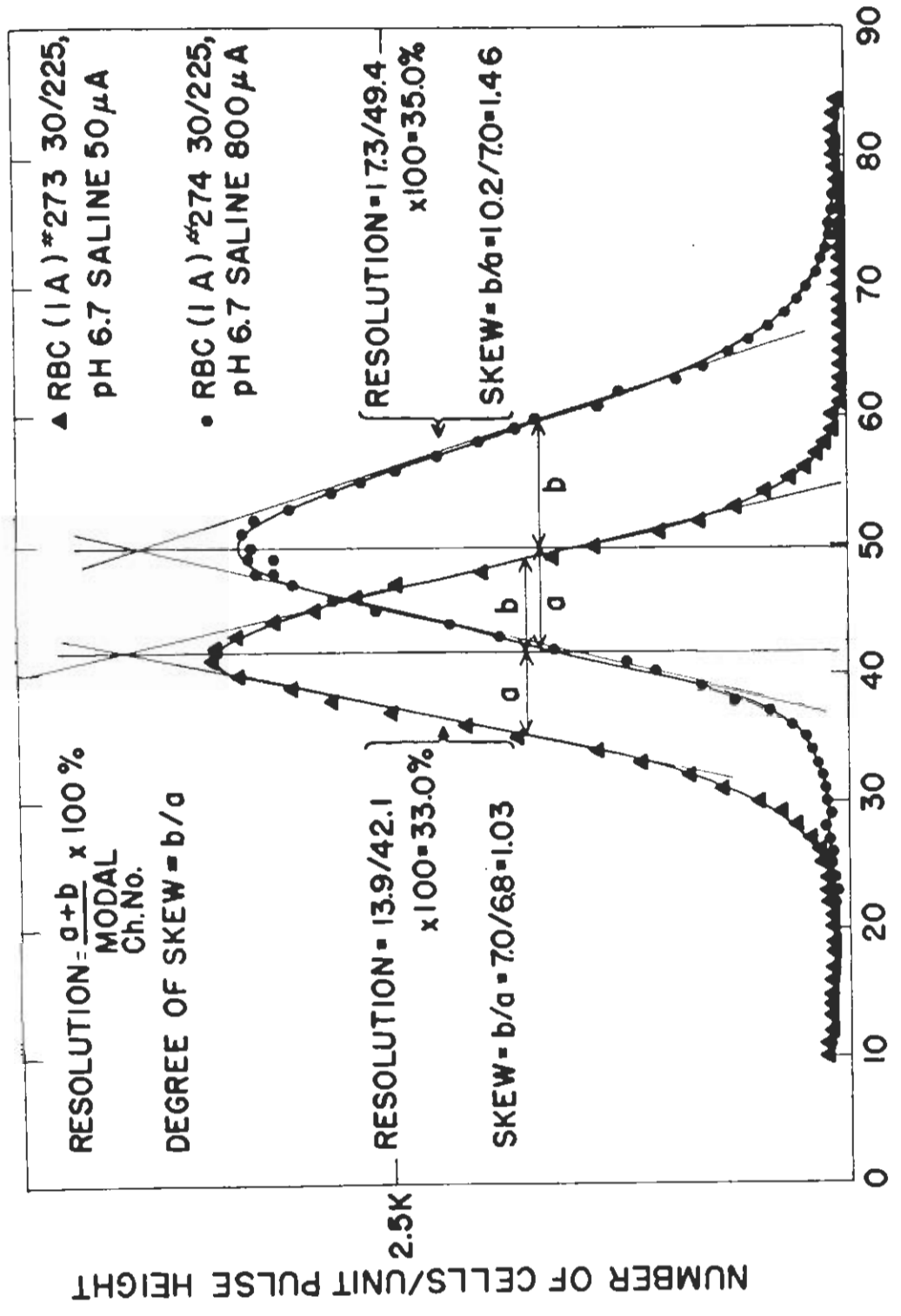

Fig. 1. Volume distribution of red blood cells of a female adult (1A) suspended in pH 6.7 isotonic saline in a 30/225 aperture at I_a = 50 and 800 μA.

We believe that the low current spectra are reasonably close to the true volume distribution for reasons of internal consistency of the data and by comparison with results of other workers using independent methods. There are several lines of approach that have been taken to the problem of red cell size:

(a) Diameter measurement microscopically (1).

(b) Diameter and thickness measurements in a microscope to yield a calculated volume (2).

(c) Ultra-soft X-ray microscopy (3).

(d) Mean cell volume measurement.

(a) Price-Jones Method

This method consists of measuring by microscope projection the diameters of human red cells in stained dry films; Price-Jones reports diameter distributions on 100 healthy adults of 500 cells per adult, or a total of 50,000 measured diameters (1). Each of the 100 cases was analyzed for mean diameter and standard deviation; the over-all mean diameter was 7.202 μ and the mean standard deviation 0.487 μ. A few of the histograms were plotted and Gaussian fits made; Pearson's chi-square test showed these Gaussian fits to be quantitatively good. The conclusion is that the diameter distribution in dry film for normal adults is approximately Gaussian. This result can be converted into a red cell volume distribution which can be compared with our data with the following simplifying assumptions:

(a) Human red cells in isotonic saline have a Gaussian diameter distribution.

(b) Mean diameter is 8.5 μ (4).

(c) Standard deviation is 0.5 μ (4).

(d) Shape is cylindrical.

(e) Thickness is constant, or

(f) Thickness is proportional to diameter.

This calculation has been made and the two resulting volume distributions (Fig. 2) are unimodal, slightly asymmetric (skewed to the right), and roughly Gaussian. For assumption e, resolution is 28 per cent and b/a = 1.11; for assumption f, resolution is 43 per cent and b/a = 1.23. Our results (Table 1) with the 30/225 aperture are intermediate (and closer to e); resolution averages 34 per cent and b/a averages 1.10. Although the cylindrical model of the red cell is oversimplified, the agreement suggests that our volume distribution in pH 6.7 isotonic saline with the 30/225 aperture is reasonable, in spite of the crenation and distortion generated by this medium. Note that the average modal volume of the same 4 adults (Table 1) is 77 μ^3; the mean value will be slightly larger and within the range of normal taken as 70 to 94 μ^3 for males and 74 to 98 μ^3 for females, mean volume 87 μ^3 for both (5).

This calculation also illustrates the fact that if one of the red cell geometric parameters (as diameter, volume, surface area, etc.) is Gaussian, then the others may not be. There is no a priori reason for expecting any particular parameter to be Gaussian; indeed, none may be. Only observational data can decide. However, if the diameter is distributed normally, volume will not be and analysis of such an asymmetric volume distribution into the sum of 2 Gaussians is valid mathematically but has no biological meaning.

More recently, Larsen (6) has used Price-Jones' technique in the study of the macrocytosis of hepatic disease and of pernicious anemia in partial remission. He also finds that the red cell diameters in normal individuals are normally distributed about the mean with a standard deviation of 0.46 to 0.50 μ (in dried smears). In his patients, however, the distribution was definitely different, and the histograms could be resolved into 2 and sometimes 3 Gaussians. Larsen feels that only one population (normocytes) is present in normal individuals. When the distribution resolves into 2 Gaussians in cases of macrocytosis, 2 populations are present (macrocytes in addition to the usual normocytes). Sometimes the distribution resolves into 3 Gaussians, in which case normocytes, macrocytes (having normal volume in vivo but which spread out to larger diameter in dry film), and megalocytes (having increased volume and hemoglobin in vivo) are present -- 3 distinct populations. Much of this work on anemias was first reported by Price-Jones (1), and more recently by Brecher (7) and others using Coulter counter techniques. The over-all conclusion seems to be that in normal people one red cell size population is present, but that certain diseases are accompanied by the presence of one or more additional populations.

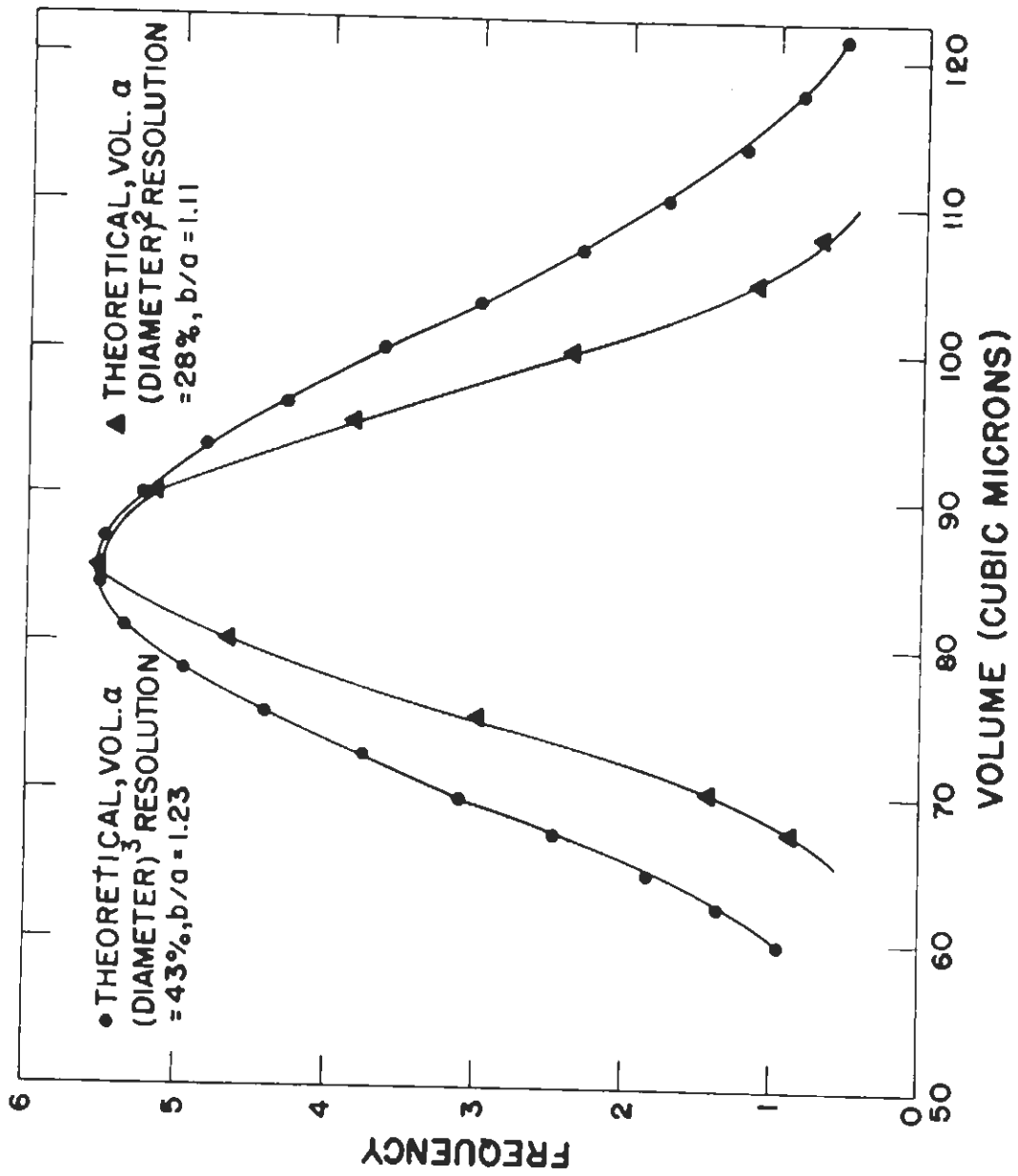

Fig. 2. Volume distributions of red blood cells calculated on the assumptions of a Gaussian diameter distribution (mean = 8.5 μ, standard deviation = 0.5 μ), and a cylindrical shape of constant thickness and thickness proportional to diameter.

(b) Rand and Burton Method

Rand and Burton (8) have calculated red cell areas and volumes from measurements of dimensions in a hanging drop preparation. Their data are published in such a way that red cell volume distribution is not obtainable; however, the authors have these data but were unable to send it in time for this publication.

(c) Henke Method

The technique developed by Henke (3) uses an entirely different approach wherein the mass of vacuum-dried individual red cells is measured by using ultra-soft X-ray absorption. The result is essentially the hemoglobin mass distribution. Henke reports this distribution for 800 red cells of one presumably normal male adult. The curve is unimodal, roughly symmetrical, and with a 34 per cent resolution. Red cells consist almost entirely of hemoglobin (~40 per cent) and water (~60 per cent). If the fraction of hemoglobin per cell is constant and this one individual is typical, then Henke's result supports our 30/225 aperture results nicely.

(d) Mean Cell Volume Measurement Method

This technique has many variations; the most common one is measurement of packed red cell volume by centrifugation and a red cell count (number per unit volume of whole blood). The uncertainty in this method is choice of proper centrifugal force and centrifugation time. We are currently working with this method.

Finally, we point out some of the weaknesses in our effort. One is the question of the diluent and its effect on red cells. Isotonic saline, either buffered to pH 6.7 or unbuffered, distorts red cell shape and crenates the surface. Eagle's solution made up following Brecher (7) seems to preserve the biconcave disc shape better. Spectra taken with this diluent are similar to those with pH 6.7 saline; therefore, perhaps the severe shape changes seen under the microscope have little effect on the measured volume distribution. It is not yet clear what is happening here and whether

other diluents may be superior. Another possible source of error is the use of polystyrene spheres for calibration, especially when diluents are used in which the cells are biconcave discs. Shape and orientation effects may be significant; experiments underway with a large-scale aperture mockup system should solve this problem.

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QUANTITY PRODUCTION OF SYNCHRONIZED MAMMALIAN CELLS IN SUSPENSION CULTURE, D. F. Petersen and E. C. Anderson. Nature (in press).

Synchronized mammalian cells in yields approaching 1 gram, representing predetermined segments of the life cycle, have been produced by reversibly inhibiting DNA synthesis with excess thymidine. When cells were followed by frequent mitotic index determinations through several successive generations, the degree of synchrony rapidly deteriorated. A genetically homogeneous clone isolated and synchronized by the same technique exhibited essentially the same decay rate as wild type cells, leading to the conclusion that the capacity to desynchronize is a fundamental nonheritable characteristic of growing cells.

SYNCHRONIZED MAMMALIAN CELLS: AN IMPROVED METHOD OF STUDY BY ELECTRONIC CELL COUNTING, D. F. Petersen and E. C. Anderson. J. Cell Biol. (submitted).

Synchrony induced in Chinese hamster ovary cells in suspension culture by excess concentrations of thymidine deoxycytidine, deoxyguanosine, and deoxyadenosine was simultaneously evaluated by electronic particle counting and conventional mitotic index determinations. Thymidine was found superior to the other deoxynucleosides as a phasing agent in terms of the quality of synchrony attained and absence of toxicity to the cells. Cell counting by electronic means provided data in good agreement with that obtained microscopically with the following advantages: (a) integral data are immediately available, making it possible to monitor a developing experiment continuously; (b) the much larger number of cells enumerated permits determination of individual experimental points within the limits of counting statistics; and (c) synchronized suspension cultures enumerated by electronic counting techniques are adaptable to chemostat operation and automated sampling at frequent intervals.

SYNCHRONIZED MAMMALIAN CELLS: AN EXPERIMENTAL TEST OF A MODEL FOR SYNCHRONY DECAY, E. C. Anderson and D. F. Petersen. Exptl. Cell Res. (submitted).

Engelberg has recently proposed a theoretical model which makes quantitative predictions for decay of synchrony in mammalian cell cultures on the basis of the assumption that