LAMS-2780
BIOLOGY AND MEDICINE
TID-4500 (18th Ed.)

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

REPORT WRITTEN: July 1962

REPORT DISTRIBUTED: November 9, 1962

BIOLOGICAL AND MEDICAL RESEARCH GROUP (H-4) OF THE HEALTH DIVISION -- ANNUAL REPORT JULY 1961 THROUGH JUNE 1962

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

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

CHAPTER 5 - CLINICAL INVESTIGATIONS SECTION 181

1. Clinical Applications of Whole-Body Counting: Determination of Thyroidal Activity from Sodium Iodide^{131} Retention Measurements with Humco II 181
   C. C. Lushbaugh and D. B. Hale
2. Clinical Applications of Whole-Body Counting: Retention of Raovin Iodine^{131} as a Measure of Serum or Blood Loss 188
   C. C. Lushbaugh and D. B. Hale
3. Clinical Applications of Whole-Body Counting: A Clinical Comparison of the Absorbability of Ferrous versus Ferric Salts in Normal Human Subjects 194
   C. C. Lushbaugh and D. B. Hale
4. Electronically Determined Erythrocyte Volumes. III. Further Observations on the Skewed Population Profile 198
   C. C. Lushbaugh, N. J. Basmann, and H. Israel
5. A Possible Mutation Causing Internal Hydrocephaly in Offspring of an Irradiated Line of Male Mice 203
   C. C. Lushbaugh and J. F. Spalding

Clinical Investigations Section Publications 209

Manuscripts Submitted 210

CHAPTER 6 - CELLULAR RADIOBIOLOGY SECTION 211

1. Calibration of an Electronic Particle Counter for Bacterial Measurements 211
   I. U. Boone and S. H. Cox

2. Sterilization of Components for Lunar Spectrometer 216
I. U. Boone, L. T. Rivera, and E. C. Anderson

3. Attempts to Obtain Synchronous Division of Escherichia Coli B and Hemophilus Influenzae 221
L. T. Rivera and I. U. Boone

4. Cultivation of Pleuropneumonia-Like Organisms (PPLO) from Tissue Cultures 224
S. Goldstein, P. C. Sanders, and I. U. Boone

5. Growth Characteristics of HeLa Cells in Agitated Fluid Medium 228
P. C. Sanders, S. Goldstein, and T. M. Gragg

6. The Differential Sensitivity of HeLa Cells to Specific and Nonspecific Intranuclear Tritium Beta Irradiation 235
D. F. Petersen, P. C. Sanders, L. B. Cole, and S. Goldstein

7. Further Studies on Biological Effects of Magnetic Fields 241
H. Foreman and M. C. Brooks

Cellular Radiobiology Section Publications 244

Manuscript Submitted 245

CHAPTER 7 - MOLECULAR RADIOBIOLOGY SECTION 246

1. Erratum 246
D. F. Petersen

2. Nucleic Acid Chromatography 248
A. Murray, E. H. Lilly, V. E. Mitchell, and D. F. Petersen

CHAPTER 1

INTRODUCTION

A number of compelling circumstances, some associated with program reorientation, interfered with the issuance of a semiannual progress report for the period July through December 1961. The present report is, therefore, an annual presentation of the Biological and Medical Research Group's activities during the period from July 1961 through June 1962.

During the past year, program reorientation and group reorganization, in keeping with the redirection of the program, were completed. It is anticipated that the Biological and Medical Research Group's activities during the next few years will proceed along the following general lines:

(a) Metabolism of Radionuclides in Mammals Using Whole Body Counting Techniques (Mammalian Metabolism Section)

Emphasis will be on establishing normal uptake, retention, excretion, and deposition parameters of radioisotopes

in mice, rats, dogs, and monkeys for the express purpose of establishing interspecies correlations for extrapolation to man. Included also will be investigations of the influence of diet, environment, age, chemical agents, and other factors on the various metabolic parameters.

(b) Genetic and Somatic Effects of Acute and Chronic Radiation Exposure (Mammalian Radiobiology Section)

Emphasis will be on effects of radiation exposures of successive generations of mice on general population statistics and effects of acute and chronic neutron and gamma ray exposure on radiation repair rates and stamina of exposed animals as indicated by stress tolerance and life span.

(c) Environmental Radiation Studies and Methods of Measurement, Including Radiations in Space (Low-Level Counting Section)

Emphasis will be on exploiting the limits of capability of the new Human Counter (Humco II) now in full operation, double 8 x 4 in. sodium iodide crystal spectrometry, potentialities of thermoluminescent materials (e.g., LiF) for methods of measuring space ambient gamma radiation, and development of procedures and codes for electronic processing of radiation and metabolic data.

(d) Beneficial Applications of Electronic and Radiotracer Techniques (Clinical Investigations Section)

Emphasis will be on applications of whole body counters and electronic cell sizing equipment to medical diagnosis and the study of clinical diseases.

(e) Cellular Level Studies (Cellular Radiobiology Section)

Emphasis will be directed toward using mammalian cells grown in agitated fluid medium and bacteria to study the effects of radiation and related factors on metabolism and growth at the cellular level. Abnormal, as well as normal, cells will be investigated using electronic cell sizing and C^{14} and H^{3} tracer methodology.

(f) Molecular Level Studies (Molecular Radiobiology Section)

Emphasis will be on synthesis of oligopolynucleotides of known base sequence and studies of their chemical, physical, and biological characteristics, investigations of chemical and enzymatic means of polymerization of oligo- and polynucleotides of known structure, development of methods for isolation and characterization of mammalian chromatin material, and eventually studies of the effects of radiation on structural organization and function of living systems at the molecular level.

A number of personnel changes occurred during the past year. Drs. Harry Foreman and Joseph A. Sayeg terminated, and Dr. F. Newton Hayes returned as Section Leader of the Molecular Radiobiology Section after a year's study in Germany. The following table of organization shows the Group personnel, their classification, and section affiliation as of the end of the present report period.

GROUP H-4

BIOMEDICAL RESEARCH

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

MAMMALIAN METABOLISM

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

Staff Members

J. E. Furchner, Ph.D.
O. S. Johnson, B.S.

Research Assistants

E. M. Abernathy, B.S.**
J. E. London, B.S.
G. A. Trafton, B.S.

Technical Staff

S. Cordova
R. Martinez

MAMMALIAN RADIOBIOLOGY

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

Staff Member

T. T. Trujillo, B.S.

Research Assistant

M. R. Brooks, B.Ch.E.

Technical Staff

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

LOW-LEVEL COUNTING

E. C. Anderson, Ph.D., Leader

Staff Members

P. N. Dean, M.A.
J. H. Larkins, B.S.*
J. D. Perrings
M. A. Van Dilla, Ph.D.

Research Assistants

D. L. Carlson, M.S.**
M. W. Rowe, B.S.

Technical Staff

A. E. Hargett

*Casual employee.

**Summer employee.

CLINICAL INVESTIGATIONS

C. C. Lushbaugh, M.D., Ph.D., Leader

Research Assistants

D. B. Hale, B.S.
G. L. Humason, M.S.

Technical Staff

N. J. Basmann

CELLULAR RADIOBIOLOGY

I. U. Boone, M.D., Leader

Research Assistants

S. H. Cox, B.A.
S. Goldstein, B.A.**
T. M. Gragg, B.S.
P. M. LaBauve, B.A.
L. T. Rivera, B.S.
P. C. Sanders, M.S.

MOLECULAR RADIOBIOLOGY

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

Staff Members

V. N. Kerr, M.A.
A. Murray, M.S.
D. G. Ott, Ph.D.
D. F. Petersen, Ph.D.
G. R. Shepherd, Ph.D.
D. L. Williams, M.S.

Research Assistants

L. B. Cole, M.S.
G. T. Fritz, B.S.
E. Hansbury, M.A.
R. E. Hine, B.S.
E. H. Lilly, B.S.

Technical Staff

P. A. Hopkins
M. Magee
V. Mitchell
M. Murphy, Jr.**

*Casual employee.

**Summer employee.

Electronically Determined Erythrocyte Volumes. III. Further Observations on the Skewed Population Profile (C. C. Lushbaugh, N. J. Basmann, and H. Israel)

INTRODUCTION

The frequency distribution curve of erythrocyte volumes has an asymmetrical skew to the right or toward larger volumes than the mode. Although in man this curve looks like a logarithmic normal curve, in the chicken the skewed portion of the population appears to be a second population of large red cells with a wide variance in size (1). On the basis of this finding in the bird, an attempt was made to fit 2 Gaussian distributed populations to the blood of various mammals and man. Two such distributed subpopulations were found in all instances. Since these populations could be electronic (2) or mathematical (3) figmentations, an attempt was made to prove their existence by differential hemolysis of the 2 populations. This seemed feasible since study of pathologic blood seemed to show that population A was senile while population B was juvenile and, therefore, relatively resistant to hemolytic processes.

METHODS AND RESULTS

Using a 100-channel pulse height analyzer with 4 memory units so that 4 consecutive frequency distribution analyses

could be made in quick succession, as described previously (1), blood samples of chicken and man were studied after addition of 0.3 ml of 0.15 per cent saponin solution to 100 ml of 1:50,000 dilution of heparinized blood in 0.9 per cent saline. The results of this experiment are shown in the accompanying figures.

Figure 1 shows 3 sequential curves at 0, 60, and 90 sec after addition of saponin to chicken blood. Curve 1 shows the usual profile of chicken blood with 2 humps. Curve 2 shows the absence of the senile population, the presence of the juvenile population, and the presence far to the left of a new, much smaller population, the nuclei of hemolyzed cells from population A. Curve 3 shows that the 2 populations were gone at 90 sec, leaving in their stead a single small population of bare nuclei. Figure 2 shows a similar typical experiment with human blood. Since the mammalian erythrocyte is not nucleated, only stromal debris of infinitely variable, increasingly smaller sized particles is seen accumulating to the left as population A first and population B second disappear under the action of the saponin.

DISCUSSION

These experiments seem to substantiate the previous mathematical division of the total distribution curves into

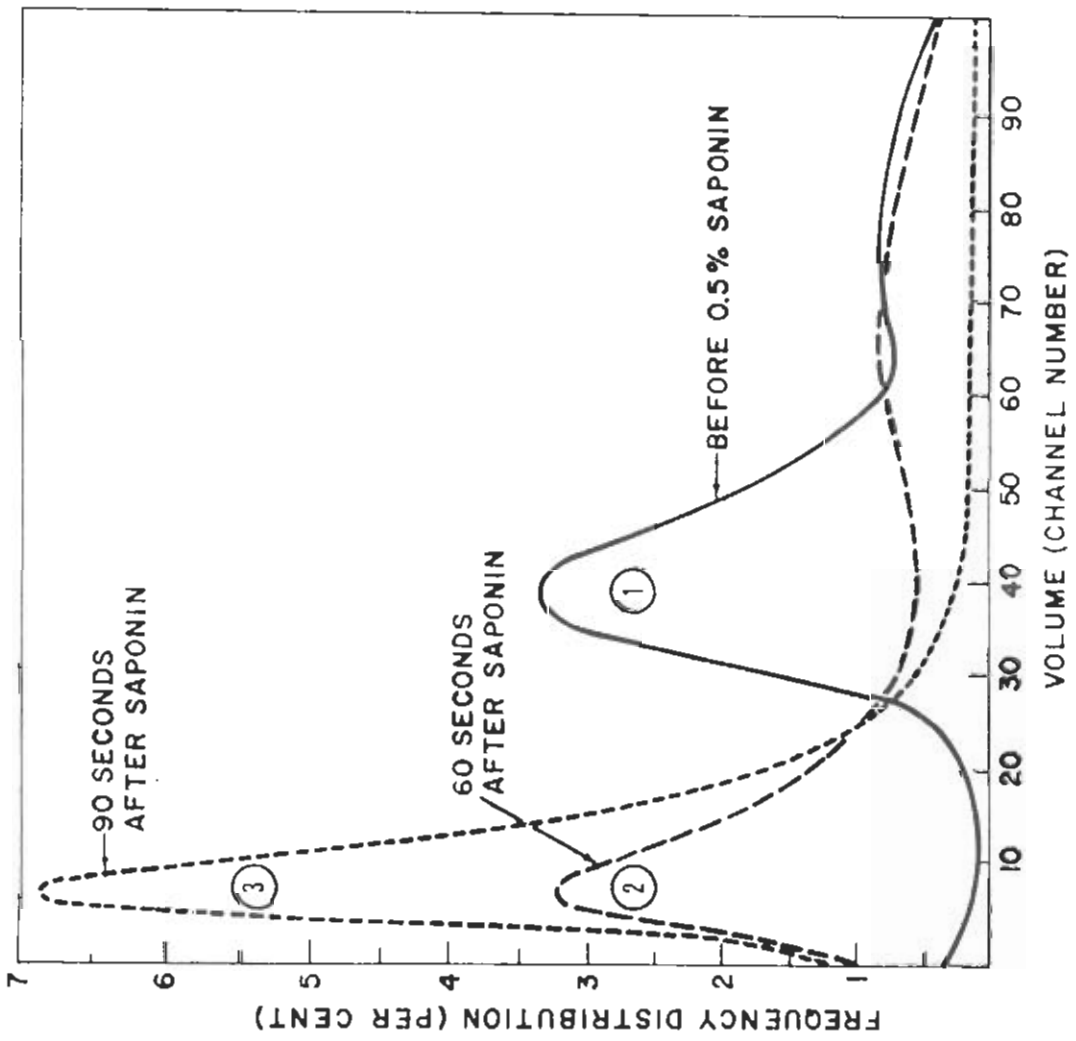

Fig. 1. Frequency distribution of cellular volumes of chicken erythrocytes before and after saponin.

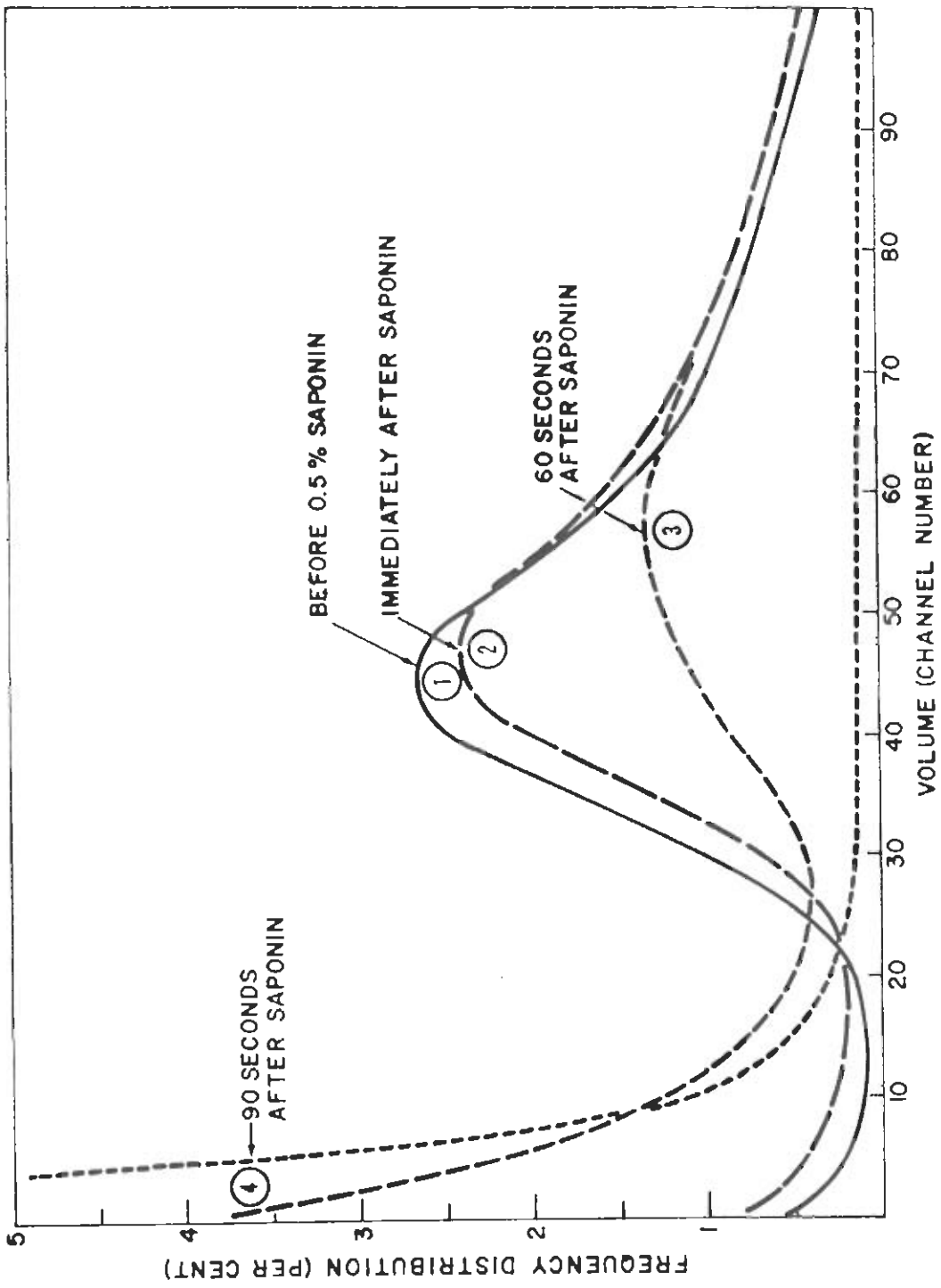

Fig. 2. Frequency distribution of cellular volumes of human erythrocytes before and after saponin.

2 populations. Further mathematical analysis is presently being directed toward making a model which will help explain the nature of population B, or young cells. Work done so far seems to show that the population B curve is a mathematical summation of an infinite number of small populations, with a large variance in volumes that are becoming smaller and more uniform as they age and shift into the senile population A.

REFERENCES

(1) C. C. Lushbaugh, N. J. Basmann, and B. Glascock, Blood 20, 241 (1962).

(2) G. Brecher, E. F. Jakobek, M. A. Schneiderman, G. Z. Williams, and P. J. Schmidt, Ann. N. Y. Acad. Sci. 99, 242 (1962).

(3) J. L. Grant, M. C. Britton, Jr., and T. E. Kurtz, Am. J. Clin. Path. 33, 138 (1960).

MANUSCRIPTS SUBMITTED

(1) C. C. Lushbaugh, A Fatal Hyperplastic Inflammatory Lesion of the Stomach of Mice Exposed to Neutrons from an Atomic Bomb, submitted to Arch. Path.

(2) C. C. Lushbaugh, A Universally Applicable Method for Assaying Thyroid Function in Vertebrates, submitted to Science.

(3) C. C. Lushbaugh, J. A. Maddy, and N. J. Basmann, Electronic Measurement of Cellular Volumes. I. Calibration of the Apparatus, submitted to Blood.

(4) C. C. Lushbaugh, N. J. Basmann, and B. Glascock, Electronic Measurement of Cellular Volumes. II. Frequency Distribution of Erythrocyte Volumes, submitted to Blood.

(5) C. C. Lushbaugh and J. Langham, A Dermal Lesion from Implanted Plutonium, submitted to Arch. Dermatol.

(6) C. C. Lushbaugh and R. L. Schuch, Clinical Use of the Arm Counter in Blood Clearance Studies, to be published in Proceedings of the Fifth Annual Symposium on Advances in Tracer Methodology, Plenum Press.

CHAPTER 6

CELLULAR RADIOBIOLOGY SECTION

Calibration of an Electronic Particle Counter for Bacterial Measurements (I. U. Boone and S. H. Cox)

INTRODUCTION

Measurements of bacterial cell number and size have always been tedious and dependent on relatively unprecise methods. The recently introduced electronic particle counter, originally designed for the counting of blood cells, has been applied to counting and to sizing of bacteria (1). Lark and Lark (2) applied the electronic counter in the study of synchronous growth of bacteria, while Toennies et al. (3) studied cell multiplication. In order to facilitate certain procedures in bacterial metabolic and genetic transformation studies, an electronic particle counter [with the Los Alamos Scientific Laboratory's pulse height analyzer system, as described by Lushbaugh et al. (4)], is being calibrated for bacterial measurements.

METHODS AND RESULTS

The counter* and pulse height analyzer have been described previously (4). Polystyrene latex spheres ranging in diameter from 0.557 to 3.04 μ were used to calibrate the instrument. The spheres or bacteria were counted in a formalized citrate-saline solution. The particle content of the diluting electrolyte was kept very low by filtering all solutions through 300-mμ millipore filters. The volume counted was 0.1 ml (28 sec) through a 30-μ diameter orifice aperture. Background counts of the diluent ranged from 200 to 1000, depending on the aperture current and threshold settings. The suspensions were diluted to count from 10,000 to 20,000 particles per 0.1 ml. The proper current control settings depended on the size of the particles studied. The "gain switch" and the "gain trim" inside the instrument were also adjusted for particle size and for size distribution. Variations in the electronic circuitry required repeated calibration and adjustments.

Calibration of the channel number (mean pulse height voltage) versus the mean particle volume is proceeding by the methods described for red blood cells (4). Typical size distribution curves for several particles studied, at the

*Coulter Electronics, Chicago, Illinois.

instrument settings indicated, are shown in Figs. 1 and 2. Preliminary bacterial counts have been performed with Hemophilus influenzae and E. coli. It is anticipated that the electronic counting techniques can be used also to detect synchronous division in bacteria.

REFERENCES

(1) H. E. Kubitschek, Nature 182, 234 (1958).

(2) K. G. Lark and C. Lark, Biochim. Biophys. Acta 43, 520 (1960).

(3) G. Toennies, L. Isgard, N. B. Rogers, and G. D. Shockman, J. Bacteriol. 82, 857 (1961).

(4) C. C. Lushbaugh, J. A. Maddy, and N. J. Basmann, Los Alamos Scientific Laboratory Report LAMS-2526 (1960), p. 372.

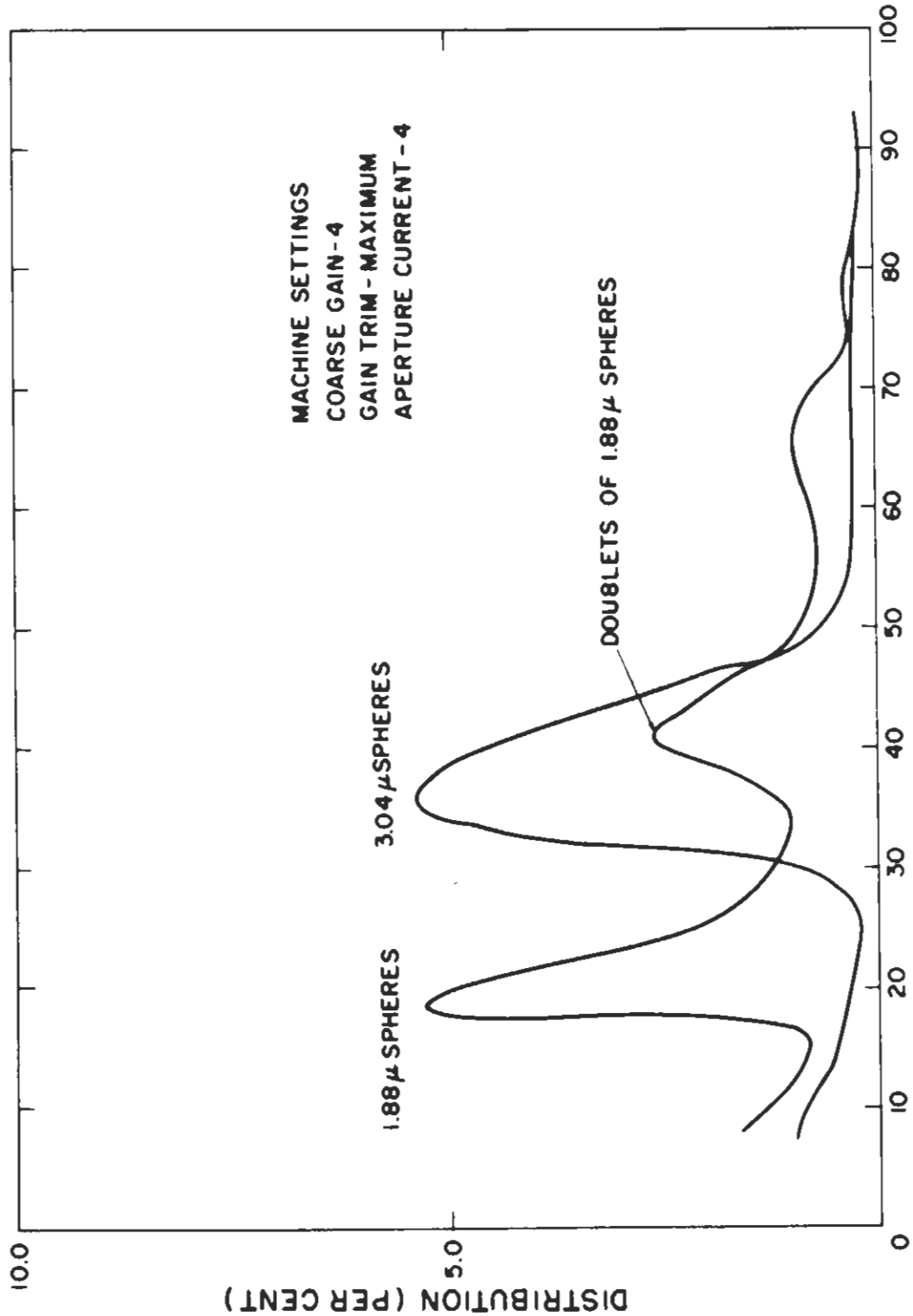

Fig. 1. Relative pulse height distributions obtained with 1.88- and 3.04-μ polystyrene spheres.

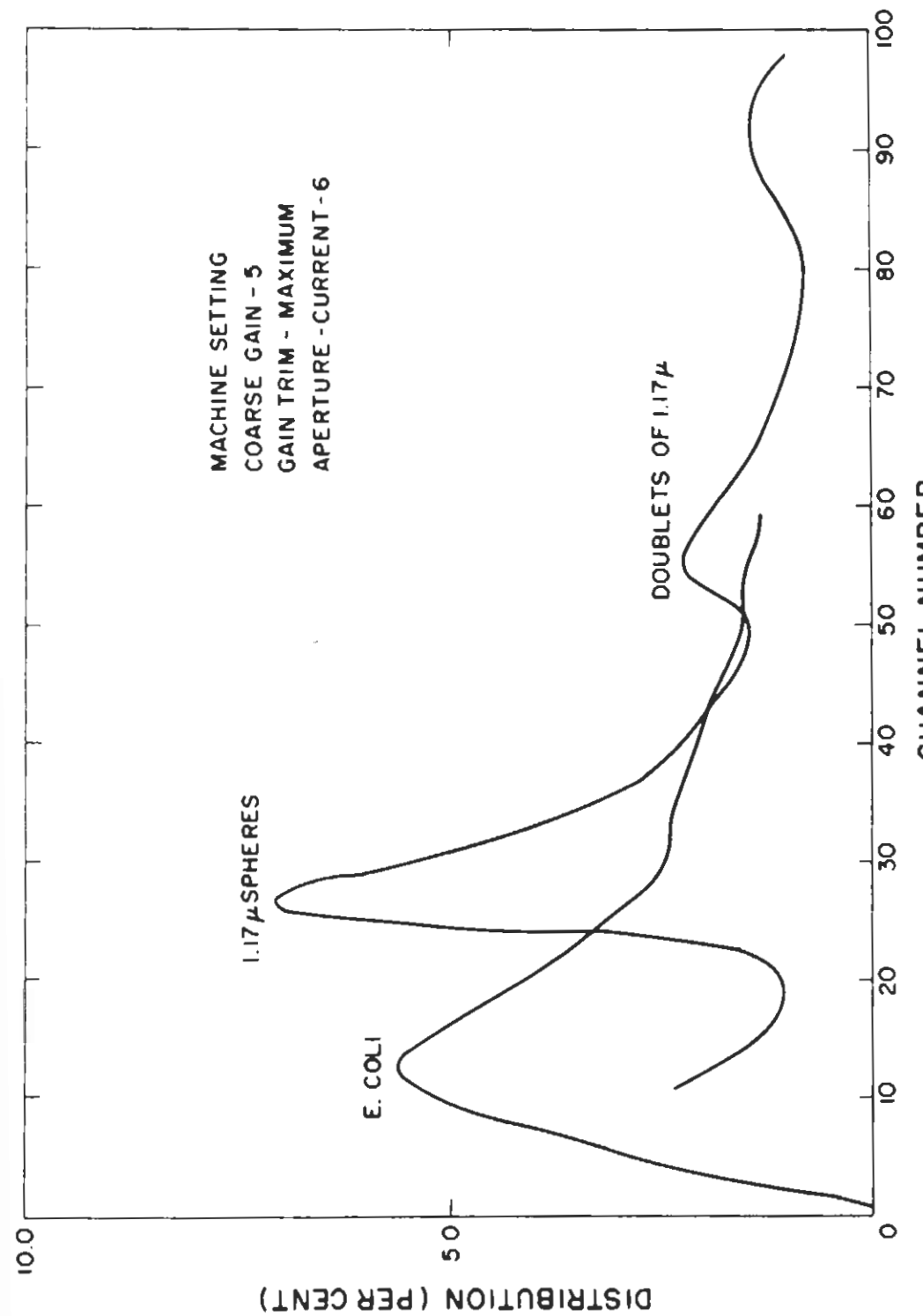

Fig. 2. Relative pulse height distributions obtained with 1.17-μ spheres and E. coli bacteria.

Growth Characteristics of HeLa Cells in Agitated Fluid Medium

(P. C. Sanders, S. Goldstein, and T. M. Gragg)

INTRODUCTION

A dependable supply of HeLa cells in the logarithmic phase of proliferation is required for biochemical and cellular studies. Several changes in the technique of growing cells in agitated fluid medium have been necessary to assure an adequate supply. A more reliable method of determining cell populations and modifications in spinner culture techniques to obtain cells in the exponential growth phase are described.

METHODS AND RESULTS

Cell Culture

The technique employed was that of growth in agitated fluid medium. Spinner flasks with a capacity of 250 and 500 ml were used. Regardless of size of flask, the initial inoculum was 2 to 4 x 10^4 HeLa cells per ml in a medium composed of equal volumes of Eagle's Basic Spinner and Puck's F4 medium (1) plus 25 γ penicillin and streptomycin per 100 ml medium, supplemented with 5 per cent inactivated horse serum. The flasks were incubated at 37°C and continuously agitated by magnetic stirrers driven by a 164-RPM synchronous motor.

Cell Growth Determination

Cell populations were determined immediately after inoculation and every 24 hr. Duplicate 5-ml aliquots of the cell suspensions were taken at each time interval and washed once with saline G (1), followed by addition of 2.5 ml of 0.05 per cent trypsin to disperse any clumps of cells that might be present. The cells were incubated at 37°C for 12 min with thorough mixing at 3-min intervals. At the end of the incubation period, 2.5 ml of complete medium was added to stop the trypsin action. A 1-ml aliquot of this cell suspension was diluted 1:50 with normal saline and counted in a calibrated Model B Coulter particle counter with the lower threshold set to eliminate debris smaller than 2275 μ^3 in volume. Each duplicate sample was counted 4 times and an average value determined. Viability of the cell suspension was determined by using a 0.5 per cent trypan blue solution as recommended by McLimans et al. (2).

Effect of Medium Change on Cell Growth

Figure 1 shows the effect of 2 additions of fresh medium on cell growth over a period of 168 hr. The line indicated by closed circles shows the growth of a culture with no medium change. Growth continued for about 72 hr with an average doubling time of about 37 hr. After 72 hr, the total

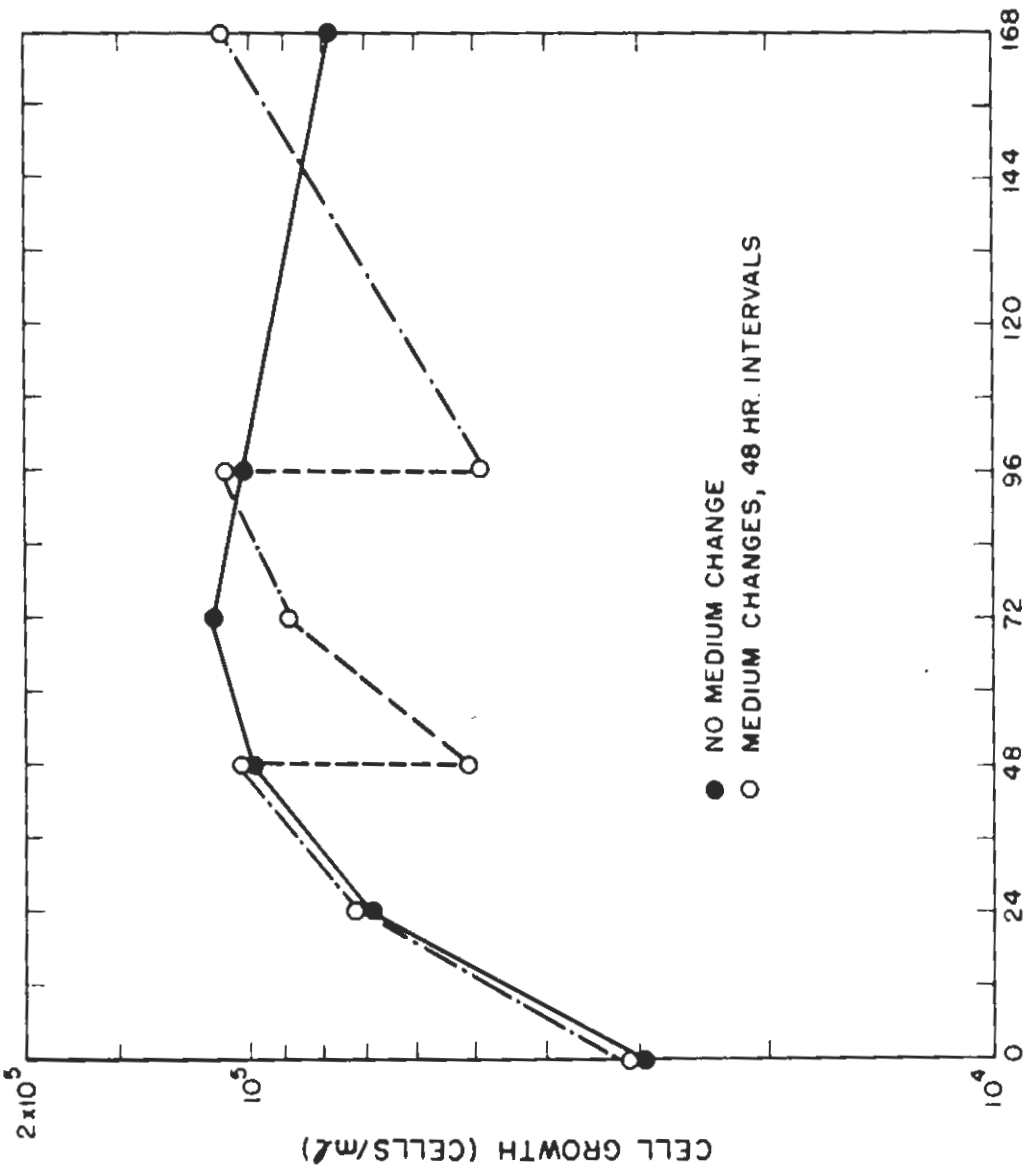

Fig. 1. Effect of 2 medium changes on growth of HeLa cells over a period of 168 hr.

cell population decreased. The line indicated by open circles represents growth of a culture kept in approximate continuous logarithmic growth throughout 168 hr by removing approximately half the cell suspension every 48 hr and replacing it with an equal volume of fresh medium. The average doubling time for this culture was about 30 hr.

The above results were sufficiently interesting that the experiment was repeated several times. A typical result obtained over a period of 240 hr with medium change every 48 hr is shown in Fig. 2. The average doubling time over the period of observation was approximately 31 hr.

DISCUSSION

The results indicated that F4, a complete medium devised by Puck and associates, promotes cell growth in a static culture. The only modification of the original formula was to eliminate the $CaCl_2$ present to prevent the possibility of cells forming large aggregates. Since this Laboratory has been using the F4-SBME mixture, no spinner flask cultures of HeLa cells have shown the characteristic "lag" phase as reported by other authors (3,4). HeLa cells grown in this medium have been maintained in a logarithmic phase of proliferation for an extended period of time by the simple process of removing a predetermined volume of cell suspension every 48 hr and adding the same volume of fresh

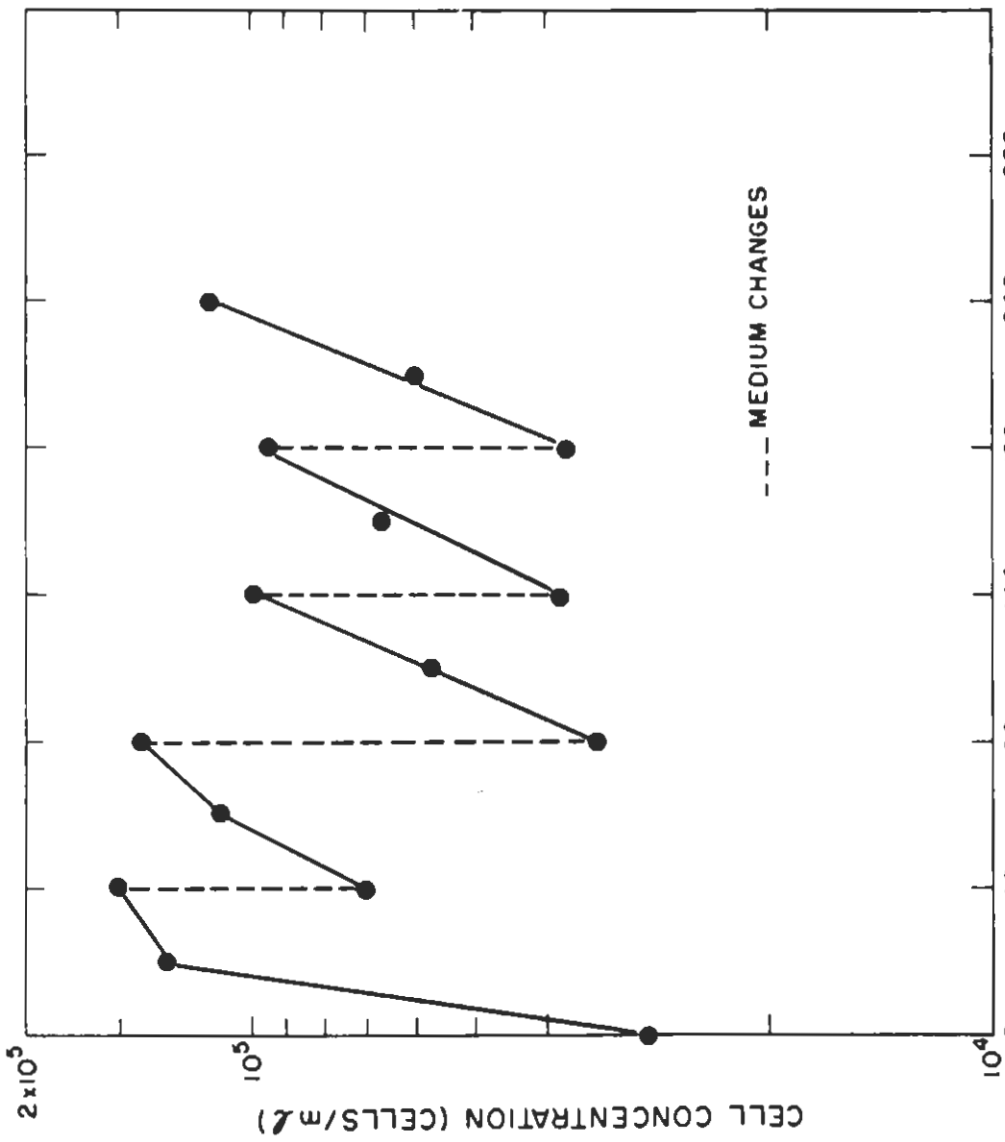

Fig. 2. Effect of 48-hr medium changes on the growth of HeLa cells over a 240-hr period.

medium to the flask. The critical factor seems to be to keep cell concentration between about 10^4 and 10^5 cells/ml. The method assures a continuous supply of healthy, viable cells for experimental work or for initiation of many new spinner flasks. Since no centrifugation is necessary to remove "spent" medium, the cells are protected from any possible trauma. This factor alone could possibly explain why the cells do not have a lag phase.

Trypsin was used in the preliminary step of determining cell populations to be certain that cells were not clumped during counting. A sample of the trypsinized cell suspension, stained with trypan blue and examined under the microscope, revealed that more than 95 per cent of the cells were viable and that approximately 1 per cent was present as double cells. A sample of the cell suspension prior to trypsinization showed approximately 10 per cent in clumps of 2 to 10 cells.

REFERENCES

(1) T. T. Puck, Department of Biophysics, University of Colorado Medical Center, Denver, Colorado, Quantitative Mammalian Cell Culture (1960).

(2) W. F. McLimans, E. V. Davis, F. L. Glover, and G. W. Rake, J. Immunol. 79, 428 (1957).

(3) J. C. Bryant, E. L. Schilling, and W. R. Earle, J. Nat. Cancer Inst. 21, 331 (1958).

(4) R. Wallace, A. Cox, and H. R. Cox, Proc. Soc. Exptl. Biol. Med. 101, 553 (1959).