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CHAPTER 1

INTRODUCTION

This report summarizes the past year's activities of the Biological and Medical Research Group at the Los Alamos Scientific Laboratory. The arrangement is by sections; however, there is considerable overlap in many instances. Work which has been published or submitted for publication has not been rewritten. Abstracts of these journal articles and manuscripts are given at the end of each section's chapter and constitute most important parts of this report.

The Biology and Medicine program includes those types of investigations which take advantage of the Laboratory's unique facilities and talents and which are of fundamental scientific significance in radiation biology. The staff and facilities are sufficiently flexible to assume without delay programmatic problems as they may arise within the Laboratory or elsewhere. Over the past few years increased emphasis has been placed on studies at the molecular and cellular levels, and interesting results from these new projects are appearing at an increasing rate.

During the next year most of these projects will continue with the total group effort remaining at about the present level. Consideration will be given to initial specific planning of facilities and experiments for the proposed biomedical research addition to the Los Alamos Meson Physics Facility.

The previous annual report of the Biological and Medical Research Group for the period July 1965 through June 1966 appeared as Los Alamos Scientific Laboratory Report LA-3610-MS (1966).

The current group organization and personnel are shown in the following table.

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to nonchilled cultures, thus providing large quantities of highly synchronized cells. From differential M. I. data, the value calculated for duration of anaphase plus telophase in CHO cells was 6.1 ± 0.7 minutes, compared to 6.3 minutes calculated from anaphase plus telophase cells present in a random population.

LIFE CYCLE ANALYSIS OF MAMMALIAN CELLS.

III. THE INHIBITION OF DIVISION OF CHINESE HAMSTER CELLS BY PUROMYCIN AND ACTINOMYCIN, R. A. Tobey, D. F. Petersen, E. C. Anderson, and T. T. Puck. *Biophys. J.* 6, 567-581 (1966).

Abstracted in Los Alamos Scientific Laboratory Report LA-3610-MS (1966), p. 64.

ORGANIC SCINTILLATION DETECTORS AND THEIR USE IN THE STUDY OF BODY COMPOSITION, E. C. Anderson. Presented at the Public Conference on Body Composition, held at the University of Missouri, Columbia (May 4-6, 1967), in press.

For the measurement of total potassium content of normal human subjects, it has been shown that large organic scintillation counters are capable of a precision of $\pm 3\%$ (one standard deviation) under routine conditions. With special care, a precision of 1% has been approached. The absolute accuracy may be comparable under favorable conditions, but the error can be larger than 8 percent.

Sources of error include counting statistics, instrument instability, contamination, and calibration errors. General methods of detecting and evaluating these errors are discussed, and some results obtained with human counters are given.

It is demonstrated that, for the measurement of ^{40}K in normal human subjects in a 4" counter, the subject's weight is the only parameter necessary to define counting

efficiency to an accuracy of a few percent and that nonuniformity of potassium distribution is not likely to cause significant errors. Methods of extending these estimates to larger animals are indicated. Animal measurements are undoubtedly more difficult because of the more serious problem with control of contamination and, in some cases, because of the larger mass.

DIFFERENTIAL RESPONSE OF TWO DERIVATIVES OF THE BHK21 HAMSTER CELL TO THYMIDINE, R. A. Tobey, D. F. Petersen, and E. C. Anderson. *J. Cell. Physiol.* 69, 341-344 (1967).

Attempts to synchronize the BHK21 hamster cell C-13 and its polyoma-transformed derivative P-183 with excess thymidine resulted in the observation that the parent cell line could be readily synchronized but the transformed derivative could not. Differences in the growth pattern indicate that excess thymidine (10 mM) stops progress of the virus-transformed derivative at all stages of the life cycle rather than exclusively in S. The data are suggestive but do not establish that the difference is a result of the presence of the virus genome.

AN EFFECT OF CELL SHAPE ON APPARENT VOLUME AS DETERMINED WITH A COULTER APERTURE, E. C. Anderson, D. F. Petersen, and R. A. Tobey. *Biophys. J.* (in press).

During the process of cytokinesis, mammalian cells in suspension culture lose their approximately spherical shape, a change which produces reduction in pulse amplitude from a Coulter aperture. Observation of the time of apparent volume reduction permits determination of the time of cytokinesis in tightly synchronized cultures obtained by selective shaking of monolayer cultures.

CELL GROWTH AND DIVISION. I. MATHEMATICAL ANALYSIS OF CELL VOLUME SPECTRA, G. I. Bell and E. C. Anderson. *Biophys. J.* (in press).

A mathematical model is formulated for the development of a population of cells in which the individual members may grow and divide or die. A given cell is characterized by its age and volume, and these parameters are assumed to determine the rate of volume growth and probability per unit time of division or death. The initial value problem is formulated, and it is shown that if cell growth rate is proportional to cell volume then the volume distribution will not converge to a time-invariant shape without an added dispersive mechanism. Mathematical simplifications which are possible for the special case of populations in the exponential phase or in the steady state are considered in some detail.

Experimental volume distributions of mammalian cells in exponentially-growing suspension cultures are analyzed, and growth rates and division probabilities are deduced. It is concluded that the cell volume growth rate is approximately proportional to cell volume and that the division probability increases with volume above a critical threshold.

The effects on volume distribution of division into daughter cells of unequal volumes are examined in computer models.

CELL GROWTH AND DIVISION. II. EXPERIMENTAL STUDY OF CELL VOLUME SPECTRA, E. C. Anderson and D. F. Petersen. *Biophys. J.* (in press).

Experimental proof is given that the volume distribution spectrum of mammalian cells in suspension culture can be determined accurately with a Coulter spectrometer. Stable spectra corresponding to the predictions of a mathematical model are observed under favorable conditions of growth. Cell volume spectrometry appears to be a useful method for diagnosing the state of

the culture with respect to past uniformity of growth rate and present population age distribution. In addition, it offers a method for quantitative study of the laws governing cell growth and division.

EFFECTS OF UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION ON THE INFECTION OF ESCHERICHIA COLI K-12 BY PHAGE- λ DNA, B. J. Barnhart and C. T. Gregg. *Virology* (in press).

Some effects of two uncouplers of oxidative phosphorylation on infection of Escherichia coli K-12 by bacteriophage- λ deoxyribonucleic acid (DNA) are described. Dinitrophenol did not interfere with the initial interaction of the cells with free DNA, and neither dinitrophenol nor carbonyl cyanide *m*-chlorophenylhydrazine affected the linear portion of the infection reaction. However, the process by which λ -DNA bound to the bacterial cell became insensitive to deoxyribonuclease was strongly inhibited by both uncoupling agents. These results support the conclusion that successful infection of E. coli with phage λ -DNA is coupled to cellular energy metabolism and localize a portion of the infection reaction which is sensitive to the uncoupling of oxidative phosphorylation. Possible energy-requiring steps in the infection process are discussed.

COMPETENCE-STIMULATING ACTIVITY IN STERILE FILTRATES FROM HEMOPHILUS INFLUENZAE, B. J. Barnhart. *Biochim. Biophys. Acta* (in press).

The physiological state of competence must develop in bacteria before the cells can incorporate extracellular DNA and undergo transformation. Results herein reported show that sterile filtrates from highly competent cultures of Hemophilus influenzae stimulate development of the competent state in cultures of low frequency competence.

CHAPTER 6

BIOPHYSICS SECTION

AN AUTOMATIC CELL COUNTER

(J. H. Larkins, J. D. Perrings, and E. C. Anderson)

Introduction

In studies with synchronous cell cultures, it is important to monitor cell concentration frequently and accurately. This can be done with a standard Coulter counter (either manually or with an automated system that duplicates the usual procedure of sample dilution, measurement, and disposal), but this is tedious and can use up a substantial fraction of the culture over a period of several days. An automatic Coulter counter which does not consume any of the culture would be very advantageous; such a counter and the problems encountered in its practical use are described below.

Methods

The basic component of the counter is a probe (Fig. 1) which dips continuously into the cell culture and which contains a Coulter aperture specially designed to minimize plugging and coincidence effects. The probe can be heat-sterilized. A measured volume of the cell suspension is drawn in through the aperture, and the number of cells in that volume is counted by the usual electronic system. The suspension is then expelled back through the aperture into the culture, and the cycle is repeated. The suspension is in contact only with inert materials (glass, silicone rubber, stainless steel, platinum).

A positive displacement pump (not

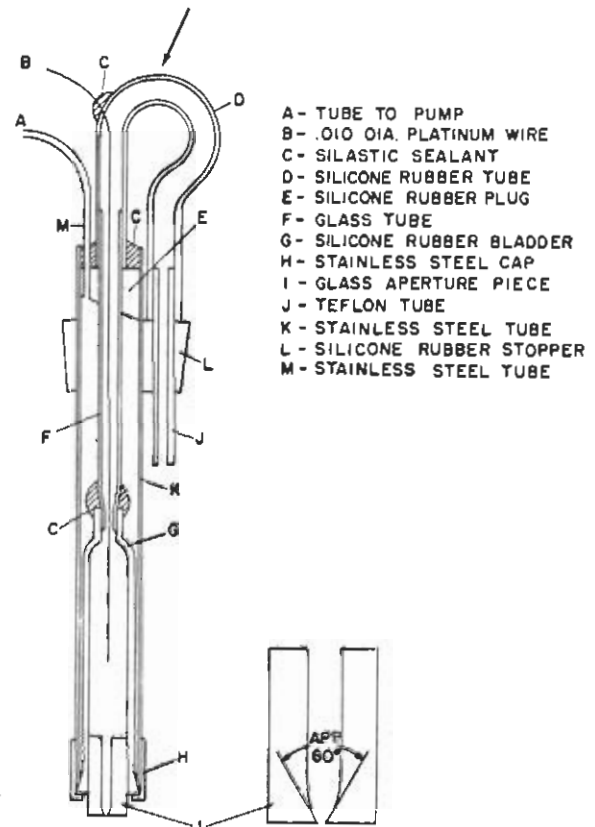


Fig. 1. Automatic cell counter probe.

shown) consisting of a glass syringe driven at a constant rate (for ratemeter output) between closely reproducible limits (for scaler readout) pumps distilled water in and out of the space surrounding the bladder. If all gas bubbles are purged from the system, an equal volume of the culture will be drawn into the bladder through the

aperture and then forced out. The volume of the bladder is about 2 cc, and the pump limits are usually set for a volume of about 1 cc. The pumping rate must be compatible with aperture size and will vary from about 1 sample per minute to 1 sample in 3 minutes.

In operation, tube D is pinched off at the point indicated by the arrow (Fig. 1) after proper manual cycling to remove gas bubbles. The exterior stainless steel shell serves as a ground electrode and the platinum wire as the active electrode.

Since no dilution of the sample is possible with this system, there is a coincidence problem at high cell concentrations. This can be minimized by reducing the effective aperture volume, and this was accomplished by reducing the effective length of the aperture by using the "knife-edge" shape shown in Fig. 1. Since the cross sectional area goes as the square of the diameter, the 60° cone gives very nearly the effect of an infinitely thin aperture, and the effective aperture volume is slightly more than that of a sphere of the same diameter. Sharp-edged apertures of this kind are easier to make than very short cylindrical apertures, since the aperture discs of the latter type are very fragile if an attempt is made to grind them less than about 200 μ thick. The sharp-edged aperture does not give as good resolution as a long cylinder but is satisfactory for gross counting. The aperture piece is made of a section of 1-mm bore glass tubing. One end is fused shut, leaving the bore terminating in a conical point. This end of the piece is ground back until the point of the cone is reached, and grinding and lapping are continued until the desired aperture diameter is reached.

Another method of shaping the inside of the piece is to use something like a dissecting needle, sharpened to the desired angle. When the glass is still soft, the smoked needle is pressed into it and allowed to remain until the glass hardens.

The cell signals were amplified and read out on a count ratemeter feeding a recorder. Since the "in stroke" gives better pulse shape than the "out stroke" (because of aperture asymmetry), a relay was used to short out the recorder during the out strokes. More accurate readout has been obtained by using a printing-scaler.

Results

Tests were made on a large-volume culture (1) at cell concentrations of up to 300,000 per cc with a 180- μ aperture. Coincidence losses were very high, but it was nevertheless possible to establish a calibration curve and to obtain results accurate to about 2 percent. The large aperture was used to minimize plugging problems. A smaller aperture would have reduced the coincidence loss and improved accuracy.

A difficulty was that the calibration curve obtained with a random culture was not identical with one for a synchronized culture. This is thought to be the result of two factors: (1) The largest factor seems to be failure of cells to separate after mitosis. The magnitude of this problem depends on the particular cell line being measured. This has the effect of a delayed response to an upturn in the synchrony wave and a reduced amplitude for the wave. (2) A smaller factor was possibly that, because of the use of a large aperture and the resulting poorer signal-to-noise ratio, the threshold level had to be set up to a point where a few small cells were producing pulses too small to register. The effect of this would, of course, be greater in the case of a synchronized culture at times when most of the cells were small.

This probe should, in principle, be very valuable for use with cultures of small volume (down to 50 ml), since no material is lost by sampling. However, in practice, two difficulties were encountered which limit its usefulness even though counting

precision attainable was very high (0.1 percent standard deviation). With small volumes of cell culture, it was noticed that rather large percentages of cells became trapped inside the bladder, apparently settling out and sticking to the top of the aperture piece. A modified probe was built with a slightly enlarged portion above the aperture with a small magnetic stirring bar in it. This eliminated the trapping.

Another effect is associated with the flow of current between two electrodes in a small volume (whether through the aperture or not). Cells are killed, and the damage seems roughly proportional to charge transfer. Silver-silver chloride electrodes were tried (to suppress such electrode reactions as generation of HOCl), but the solubility product of silver chloride was not small enough to keep the concentration of silver ion below the toxic level. Platinized platinum electrodes were tried, but the current densities (aperture current 50 to 100 μ a) were too high to maintain a reversible hydrogen electrode, and toxic electrode products were still generated. The problem is still unsolved. It would appear that a factor of 10 to 100 reduction in aperture current would make the effect insignificant for runs of up to 48 hours. This would necessitate a corresponding increase in amplifier gain with many attendant difficulties. The use of this counting method might also be extended to smaller volumes in cases where less frequent counts can be tolerated. In such cases, the aperture current could be turned off between counts, and the cell damage would be considerably reduced.

Conclusion

Within the limitations noted above, the method works well with large volumes (6 l. in the case mentioned). With a few refinements, it should work down to 1 l. By using the smallest practical aperture (which permits reducing aperture current) and other means to reduce noise, it might be possible

to operate with volumes as low as 200 cc. However, in the case of smaller volumes and long experiments, one would be approaching the limits of present design.

Reference

- (1) L. R. Gurley, this report, p. 56.

THE FLUORESCENT CELL PHOTOMETER: A NEW METHOD FOR
THE RAPID MEASUREMENT OF BIOLOGICAL CELLS STAINED WITH FLUORESCENT DYES

(M. A. Van Dilla, P. F. Mullaney, and J. R. Coulter*)

Introduction

Fluorescent staining is a widely used technique in many types of investigation of both normal and pathological cells and sub-cellular components. The usual visual detection method with the fluorescence microscope is slow and often quite subjective. A fluorescent cell photometer is being developed which quantitatively measures the fluorescent light emission of individual dyed cells at high speed, typically 10^4 to 10^5 per minute. It is expected also to provide cell size information via small angle light scattering and, in addition, act as a new sensor for cell sorting. Preliminary results have been obtained with inert particles and mammalian cells treated with the fluorescent dyes fluorescein and acridine orange. Fluorescence intensity distributions have been compared with volume distributions taken with a Coulter spectrometer. Cell counts on the same sample obtained by both methods agree within a few percent, indicating that every fluorescent particle is being sensed.

Methods

The fluorescent cell photometer is, in principle, similar to a conventional photoelectric fluorometer with the exception that instead of a d.c. measurement of a fluorescent solution we line up cells stained with a fluorescent dye, flow them one at a time at high speed across the exciting

light beam, and measure the amount of fluorescent light emitted by each cell. The arrangement is shown in Fig. 1.

The light source is a PEK-110 compact arc mercury vapor lamp mounted in a Tech/Ops Model 580-12 lamphouse. Two Bausch and Lomb microscope objectives (f/1, 32 mm, f/3) form a condenser of unity magnification; a second pair of similar Gaertner objectives form a projector, again of unity magnification. Light from the mercury arc is imaged on a slit 125μ wide by the condenser. The slit image in turn is projected on the cell stream in the flow chamber. A Zeiss BC-12 blue filter transmits light in the 3500- to 5000-Å band only; this is the exciting light. Fluorescent emission at longer wavelengths from a cell is collected by a pair of Simpson f/1.6 projection lenses at unity magnification and transmitted by a yellow filter (Corning 3-69) to the photocathode of an ITT multiplier phototube (type FW-130). The light pulse thus generated is

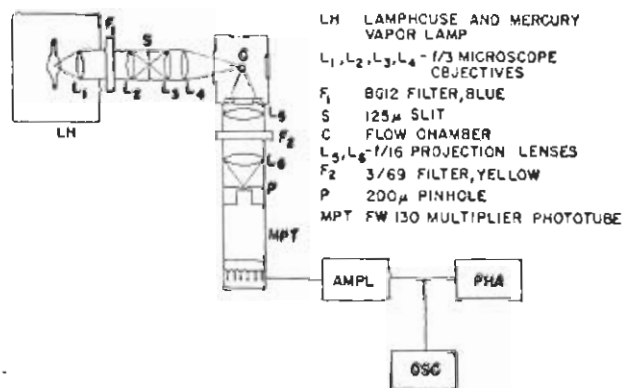


Fig. 1. Block diagram of fluorescent cell photometer.

*LASL Shops Department.

amplified and analyzed for pulse height by a Victoreen SCIPP multichannel analyzer, the result being the distribution of fluorescent light intensity for the cell population examined (i.e., distribution of dye content).

One of the crucial parts of this system is the flow chamber (Fig. 2), which follows the design used in the Vickers Instruments J12 cell counter which, in turn, follows the earlier design of Crosland-Taylor (1); the basic idea is found in a paper on laminar and turbulent fluid flow by Reynolds (2) published in 1883. A laminar flow of any convenient liquid (i.e., water, saline, sucrose solution) is established in the cylindrical bore section of the flow chamber [labeled (1) in Fig. 2, diameter 3.2 mm]. The flow then enters a smooth transition region to a short cylindrical section of smaller bore [labeled (3) in Fig. 2, diameter 0.5 mm]. The fine hypodermic tubing (internal diameter 0.5 mm) on the axis of the large cylindrical bore serves to introduce the cell suspension smoothly into the faster flowing fluid. In this way, the sample stream flows along the axis of the chamber without mixing with the main or "sheath" flow. The Reynolds number in section (1) of the flow chamber is typically 200 and well below the critical value of about 2000 which is the "boundary"

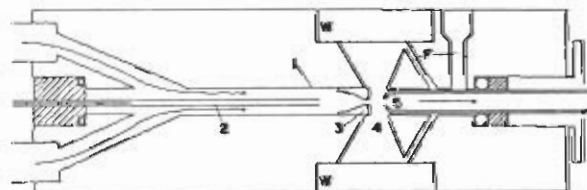


Fig. 2. The flow chamber (scale 4 times actual). W = window; F = flushing system for bubble removal; 1 = sheath flow; 2 = sample injection; 3 = nozzle; 4 = quiescent (viewing) region; and 5 = exit tube.

† In Reynolds' classic work, he introduced colored water into clear water to visualize the onset of turbulence.

between laminar and turbulent flow. In the small bore region of section (3), the Reynolds number is typically 1000 and is thus closer to the value at which turbulence can develop. The effect of the constriction is to decrease the diameter of the cell suspension stream to about 75 μ . At a typical cell concentration of 30,000/ml, the average separation of cells is 1 cm, and the chance of two cells passing a given point simultaneously is very small. Thus, the cells have been lined up for exposure to the exciting light one at a time.

The flow jets out of the nozzle (3) across a relatively quiescent region (4) and exits via the tube (5), of the same internal diameter as the nozzle outlet. Laminar flow is maintained across the quiescent region. The flow chamber is of square cross section with 4 windows, allowing a perpendicular view of the fluid flow. Exciting blue light enters through one window, and the fluorescent emission at 90° is viewed through another window; the cone angles allow use of f/1 optics.

In this way a cell stream of very small diameter can be generated with much larger tubes, virtually eliminating plugging problems. With the windows far from the cell stream, light scattered by dirt on windows cannot enter the detector, and the effect of multiple reflections and window fluorescence is minimized.

A pressure differential of 5 in. of mercury is maintained across the chamber, producing a sheath flow of 30 ml/min. The sample rate of 0.5 to 1 ml/min is produced by gravity flow. The cell suspension is replaced with ink for alignment purposes.

The image of the arc produced by the illumination system is focused on the sample stream as it crosses the quiescent region. The illuminated and sensed volume (sensitive volume) is then a cylinder of diameter 75 μ and height 125 μ . Cells pass through this sensitive volume one at a time at a rate of 10^4 to 10^5 cells/min with a transit time of 30 μ sec. Typical cells

have diameters of 10 to 20 μ and, therefore, an individual cell occupies less than 1 percent of the sensitive volume. Other fluorescent materials contained within the sensitive volume, in addition to the cell of interest, can give rise to a noise signal. It is, therefore, advantageous to reduce the sample stream diameter and to narrow the optical slit used in the illuminating system, cellular dimensions being the lower limit. Part of the present effort is in this direction.

The multiplier phototube must be sensitive to long wavelength light ($> 5000 \text{ \AA}$), suggesting the S-20 type photocathode. In addition, low noise is crucial because of the low light levels being sensed. Tests of several multiplier phototubes indicated that the ITT Model FW-130 was best. The very small effective diameter of the photocathode (2.5 mm) greatly reduces thermionic and sidewall noise. In addition, the effective photocathode can be moved about on the actual (and larger) photocathode by magnetic deflecting coils, greatly aiding line-up of the system. Fluorescent light from the sample stream is focused by unity magnification optics on a 200- μ pinhole just in front of the photocathode. In this way the field of view of the FW-130 is limited so that stray light from outside the 75 x 125- μ sensitive volume is minimized.

The dyes used (acridine orange and fluorescein) absorb strongly between 4000 and 5000 \AA with an absorption maximum near 4900 \AA . The fluorescence emission is contained in a wide band extending from 5000 to 6000 \AA . The brightest conventional sources of 4000 to 5000 \AA light available are the short arc mercury vapor lamps such as the PEK-110 with an electrode separation of 300 μ and a brightness of 140,000 candles/cm². Since image brightness cannot exceed object brightness (3), this represents an upper limit on cell stream illumination with conventional sources. If f/1 optics are used to collect the light from a point source placed at the focus,

1/16 of the emitted light will be collected, and this represents a practical upper limit on aperture.

Results

The first positive results were obtained with ragweed pollen stained with acridine orange. These particles are relatively uniform in size and pick up the dye quite effectively. They are about 20 times as bright as CHO cells treated with fluorescein diacetate (see below). With this relatively crude first experimental arrangement, fluorescent particle signals were barely 2 times noise level. With the improved current apparatus, the same fluorescent particles give signals about 1000 times above instrumental noise. The pulse height distribution of the fluorescent ragweed pollen is shown in Fig. 3, along with the light scatter distribution (BG-12 and 3/69 filters out) and the volume distribution (measured with a Coulter spectrometer). The amount of light scattered per particle is much greater than the fluorescent emission per cell under these conditions. All three distributions are similar in shape -- approximately Gaussian. The widths of these distributions halfway down from the peak expressed as percent of the mode are given in Fig. 3. Note that the scatter and the

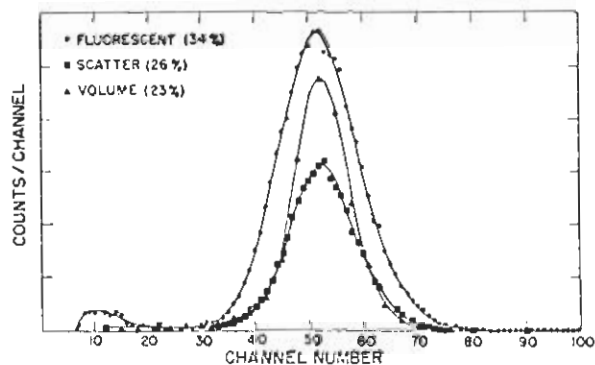


Fig. 3. Fluorescent, scatter, and volume spectra of acridine orange-stained ragweed pollen. Values in parentheses are width halfway down from peak as percent of mode.

fluorescent distribution are broader than the volume distribution; they are expected to be narrower by close to 33 percent if scatter and fluorescent light intensities are proportional to particle surface area and if the particles are spherical. The broadening may be due in part to instrumental factors (like non-uniform illumination of the particle stream) and in part to the knobby surface of the ragweed pollen. Microscopic examination of sections shows that the acridine orange remains near the pollen surface.

From sample flow rates and areas under the curves of Fig. 3, particle concentrations have been calculated and are listed in Table 1. All these values are within a range of ± 3 percent, showing that all methods agree and all particles pick up the dye and fluoresce.

In addition to these results on pollen, we have measured fluorescence distributions of Chinese hamster ovary (CHO) cells showing fluorochromasia (4). CHO cells were added to saline solutions of 10^{-5} M, 2×10^{-6} M, and 5×10^{-7} M fluorescein diacetate (FDA) and remained in these solutions during the course of an experiment. The FDA, which is not fluorescent, is quickly absorbed into the cells where it is hydrolyzed to fluorescein by enzyme activity. Fluorescein, the fluorescent product of this reaction, accumulates within the cells. Cells treated in this manner appear green against

a dark background in the fluorescent microscope. Their fluorescence is weaker than the ragweed pollen stained with acridine orange by a factor of 15 to 50, but is sufficient to yield the distributions shown in Figs. 4 and 5. These fluorescent intensity distributions were taken under different electronic gain, and hence the three modal channels do not fall as closely together as indicated in the figures. If the modal channel of the 10^{-5} M FDA sample is taken as 28, then the modal channels for the other two samples at the same gain are 16 (2×10^{-6} M FDA) and 11 (5×10^{-7} M FDA).

TABLE 1. CONCENTRATION OF A SUSPENSION OF RAGWEED POLLEN STAINED WITH ACRIDINE ORANGE AND MEASURED BY FLUORESCENCE, SCATTER, AND VOLUME SENSORS

Method	Particles/ml
Fluorescent cell spectrometer	10,200
Light scatter	10,700
Coulter volume spectrometer	10,070
Coulter counter	10,300

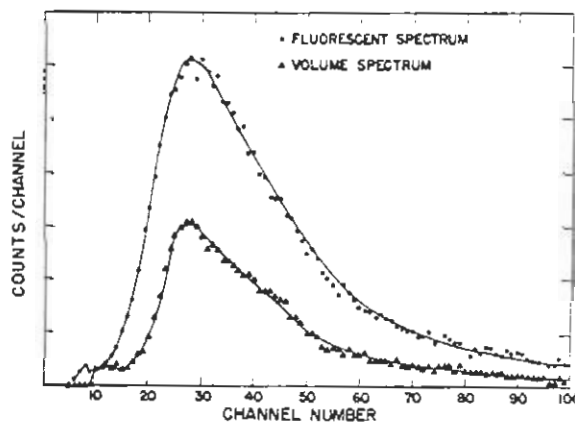


Fig. 4. Fluorescent and Coulter spectra of CHO cells treated with 10^{-5} M fluorescein diacetate.

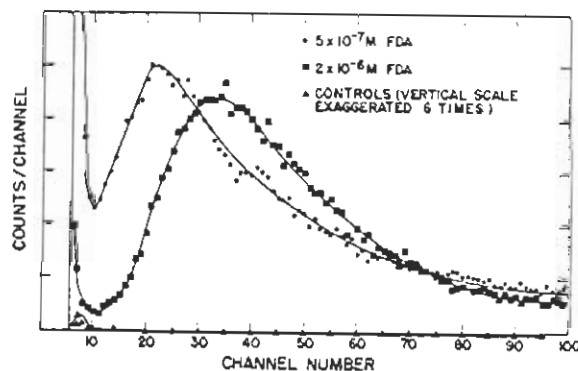


Fig. 5. Fluorescent spectra of CHO cells treated with 2×10^{-6} M and 5×10^{-7} M fluorescein diacetate and controls (see text for explanation of electronic gain used).

Thus, the fluorescent signal strength is not proportional to FDA concentration but tends to saturate. If no FDA is added to the cells, there is virtually no fluorescent signal present. At all three FDA concentrations, there was low channel fluorescent noise present which increased with time and which was more prominent relative to cell fluorescence at the lower FDA concentrations. If the fluorescent CHO cell suspensions are centrifuged at 3×10^3 RPM for 5 minutes in a clinical centrifuge and the supernatant liquid examined with the fluorescent cell photometer, the low channel noise persists and the cell signal is absent. Thus, the noise appears to be a fluorescence of approximately constant intensity, possibly from debris saturated with fluorescein or fluorescein in solution.

A typical volume distribution is shown in Fig. 4. Both volume and fluorescence distributions show little or no change in shape or modal value over a 50-minute period, but both show a similar drop in area (i.e., cell concentration) with a half-life of about 2 hours. This indicates no loss in fluorescence, rather a loss of cells by sticking to glass, disruption, or some other mechanism. The similarity in shape of the volume and fluorescence spectra indicates that cell fluorescein content is proportional to cell volume.

Discussion

The ragweed pollen results show that the fluorescent light distribution and fluorescent particle count can be measured at high speed (about 10^4 /min) for brightly fluorescing particles with good accuracy. The fluorescent distribution may be somewhat too wide due to instrumental broadening effect, but this can be investigated and may actually be real. The CHO cells containing fluorescein are probably as bright as most biological cells treated with fluorochromes; they yield signals

which are well above instrumental noise, although background fluorescence (probably from fluorescein in solution or fragments) is a problem. CHO cells stained with acridine orange show a similar background fluorescence. Two approaches to this problem are being tried: better staining methods and improvement in instrumental design. The latter approach includes reduction in sensitive volume and pulsing the fluorescence sensor.

As the sensitive volume is reduced, solution fluorescence noise riding on a cell signal is reduced. Cell stream diameter can be reduced by increasing sheath flow at constant sample flow while maintaining laminar flow. These factors are being investigated. Light beam diameter can be reduced by reducing slit width with no change in light intensity. This represents an advantage but an increasing waste of light, something inherent in conventional light sources and optical elements (i.e., lenses and mirrors). A better solution would be use of a laser beam, because the entire light output of a laser can be focused down to image dimensions equal to (and even below) minimum useful cell stream diameter. Fortunately, argon ion lasers with large outputs (1 to 2 watts) in the 4000- to 5000-Å band have recently become available. This power is actually comparable to the output of the PEK-110 mercury vapor lamp in the same band; the difference lies in the ability to concentrate it. We plan to use such a laser (Spectra-Physics Model 140) as the light source. Two to three orders of magnitude more cell stream illumination is expected. The output beam (diameter about 1.2 mm) can be focused to a "slit" image with a pair of cylindrical lenses. Although the light intensity on a cell is very large under these conditions, it can be shown equivalent to a 30-second exposure in a Zeiss fluorescence microscope using 200-watt mercury vapor lamp illumination. Theoretical and experimental evidence indicates no dye saturation.

Since cells are present in the sensitive volume about 1 percent of the time, only noise pulses are counted during the remaining 99 percent of the time. These can be eliminated and two additional advantages gained by converting to pulsed operation in which the system is turned on only when a cell is in the slit. We plan to use light scattered at small angles as a trigger signal to open a normally closed gate to the analyzer, thus collecting fluorescence pulses only when a cell is present. In this way we hope to reduce noise pulse accumulation in early channels, generate a time-coincident scatter signal which should yield information on cell size, and use this signal to permit fluorescent signal retrieval from noise by averaging.

It is also possible that the present rather inefficient fluorescent light collection may be improved by use of reflecting chambers and appropriate optics in place of the present f/1.6 optics. Several designs, including parabolic, elliptical, and spherical reflectors, have been investigated with the aid of lens-design computer codes. Image quality is very poor for the first two, fair for the spherical case. Due to uncertainty at present as to allowable image deterioration and also to fabrication difficulties, this approach is postponed until it is clear that more efficient light collection is necessary.

Crude spectral analysis is also contemplated for the future. A second FW-130 multiplier phototube at the other 90° window would sense the same fluorescence signal as the present FW-130. With appropriate filters one could sense part of the emission band, and the other could sense the rest of the emission band.

Acknowledgments

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BIOPHYSICS SECTION

PUBLICATIONS AND ABSTRACTS OF MANUSCRIPTS SUBMITTED

VOLUME DISTRIBUTION AND SEPARATION OF NORMAL HUMAN LEUCOCYTES, M. A. Van Dilla, M. J. Fulwyler, and I. U. Boone. Proc. Soc. Exp. Biol. Med. 125, 367-370 (1967).

Abstracted in Los Alamos Scientific Laboratory Report LA-3610-MS (1966), p. 265.

INTERCOMPARISON OF THE RELIABILITY OF BODY ^{137}Cs MEASUREMENTS ON HUMAN BEINGS, C. J. Maletskos, P. N. Dean, S. A. Lough, and C. E. Miller. U. S. Atomic Energy Commission, Division of Biology and Medicine, Report TID-23740 (June 6, 1967).

As a result of large variations in the ^{137}Cs body burdens reported by the participating laboratories, the Division of Biology and Medicine, USAEC, appointed, in 1962, an ad hoc committee to conduct an intercomparison between these laboratories on the reliability of body ^{137}Cs measurements on human beings.

The intercomparison was divided into three phases: 1) intercomparison with National Bureau of Standards ^{137}Cs sources to determine variations in the absolute activity of the laboratory standards and to place the laboratories participating in this study on a common basis for future work; 2) determination, at three different distances from the detector, of the ratio between three ^{137}Cs sources with nominal activities of 1000, 100 and 10 nCi (i.e., between a decade set) to assess the ability of each laboratory to account for all the physical and electronic variables in measurements conducted with whole-body counters; and

3) determination of the ^{137}Cs body burdens of two well-documented subjects (one with a "normal" burden and the other with a burden ~10 times greater) by the usual procedure of each laboratory to assess the reliability of calibration factors and the control of physiological and other physical variables. Each laboratory (16 of the 17 responded) supplied information in the same form, in order to make the evaluation consistent and convenient.

Each participating laboratory compared the old value of its working standard to a new value based on reference to the certified NBS standard. The average of the ratios of the new to old value was 1.01 ± 0.04 . The ratios of the participating laboratories' values for the decade sources to the corresponding Argonne National Laboratory values averaged 1.00 ± 0.01 , for each of three source to detector distances. The ratios of the body burdens found to the values reported by the Los Alamos Scientific Laboratory averaged 0.96 ± 0.11 for the high ^{137}Cs burden and 0.93 ± 0.13 for the low ^{137}Cs burden. Averages of similar ratios for body potassium were 0.93 ± 0.08 (high burden) and 0.90 ± 0.06 (low burden). The conclusion is that these laboratories can be capable of reliable ^{137}Cs and K body measurements in human beings. Differences which occur in such body measurements appear to be associated with physiological variables (affecting calibration factors, for example) rather than with physical or electronic variables.

In view of these results and of the time it has taken to complete this study, it is not clear why the original results

were so variable. Since that time, the "normal" ^{137}Cs burdens have increased, instrumentation has improved, a greater attention has been devoted to calibration factors, and each laboratory has gained much experience. Any and all of these factors have probably contributed to the state of the art which has been demonstrated by the intercomparison.

WHOLE-BODY NMR SPECTROMETER, J. A. Jackson and W. H. Langham. Submitted to Rev. Sci. Instr.

A low-frequency NMR spectrometer has been built that will accommodate sample volumes of 0.2 to 1.25 l. A shielded solenoid provides a uniform field of about 10 G. Signals are detected by a symmetrical rf bridge whose coils are bank-wound of litz wire with $Q \sim 300$ to 400 at 40 kHz. In the smaller coil, homogeneity is sufficient to distinguish signals of protons in protein from those in fat and water. Proteins show a broad resonance underlying the fat and water resonance which is not clearly resolved. The broad resonance is identified with protein by spectra of pure H_2O (sharp peak only) and gelatin made with D_2O (broad peak only). Protein hydration water is believed to be included in the main water peak. Lard is used to identify the signal from fat. Some unidentified lines are occasionally seen. Spectra have been obtained from gelatin, whole eggs, egg whites, egg yolks, and dead mice. In addition, what is believed to be the first NMR signal ever obtained from a whole living animal has been taken on an anesthetized rat. Potential application to analysis of living animals into their whole-body composition is discussed, as is detection of signals from other nuclei.