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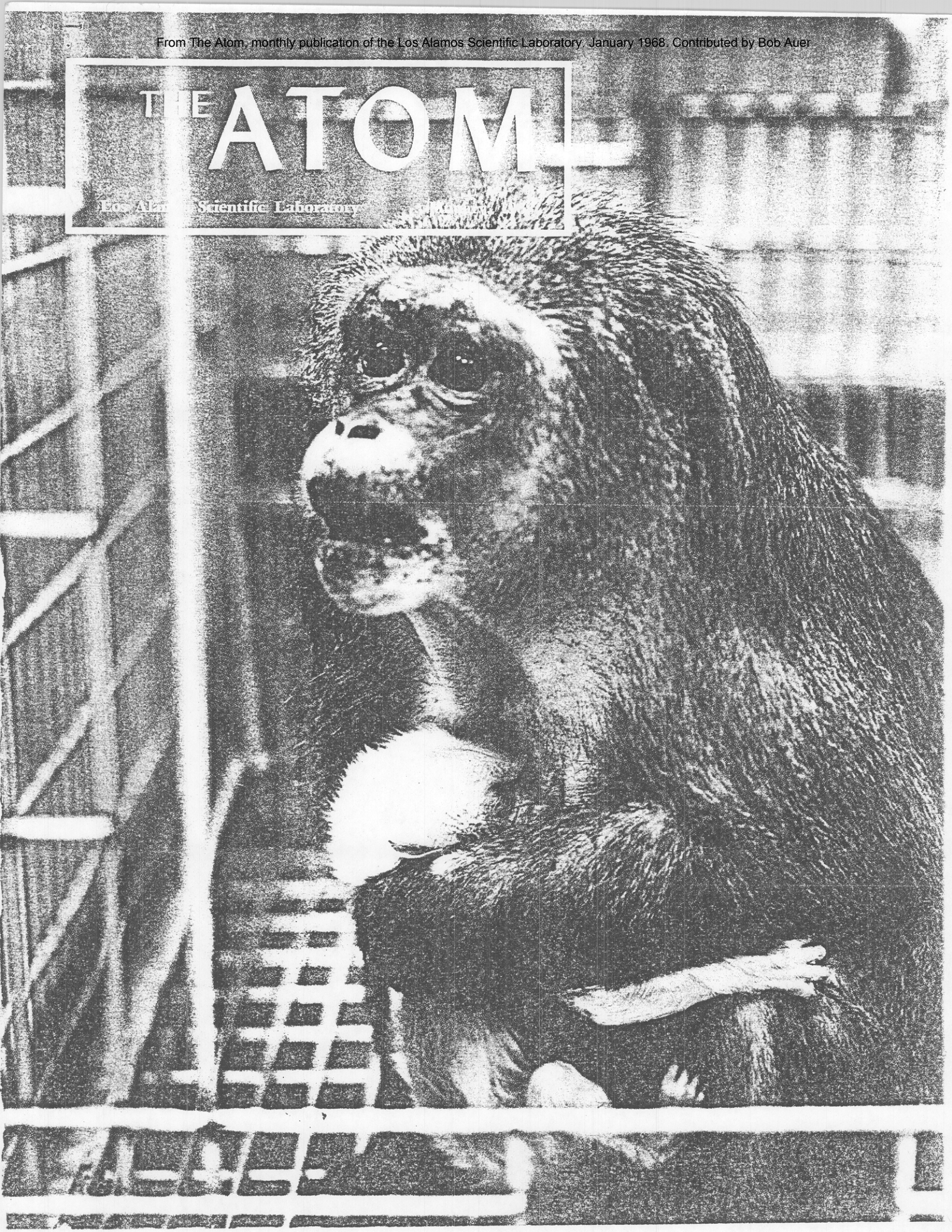
THE ATOM

Los Alamos Scientific Laboratory



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COVER:

Sixty hours after birth, Chancy slept through her first official portrait sitting for Pub photographer Bill Jack Rodgers. The mother, Nancy, produced the apparently normal offspring in spite of having received a large radiation dose in a LASL experiment. Story begins on page 7.

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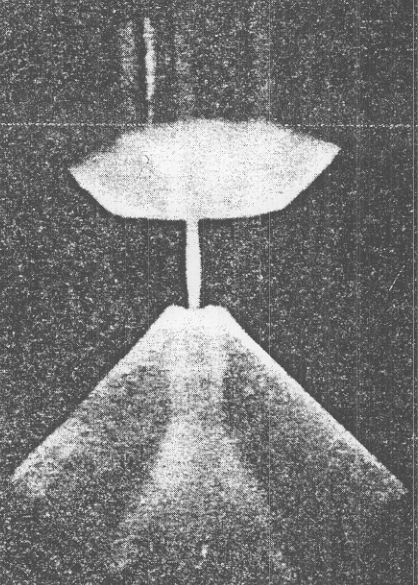

New Tools For H-4 Biomedical Research

By Bob Masterson

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IN THE EARLY 1930's a British scientist named Cecil Price-Jones and his co-workers spent about a year measuring the diameters of 50,000 red blood cells (500 each from 100 different patients). In so doing he obtained the first really comprehensive picture of the size distribution of blood cells and achieved a degree of immortality—hematologists still call cell-size distribution curves "Price-Jones curves."

The number of cells measured in a year by Price-Jones is just the number of cells that can be measured *per minute* by an electronic cell volume spectrometer in use by the biomedical research group (H-4) of the Los Alamos Scientific Laboratory. This device has been developed into a separator which not only measures the cell volumes but also sorts the cells according to size.

The separator is one of the instruments being developed by the biophysics section of H-4 as part of their general program of developing measuring and counting instruments and applying computer techniques to provide faster and better means of acquiring and analyzing biomedical research data. These instruments have potential clinical as well as research applications.

Wright Langham, H-4 group leader, has been active in the program of instrument development. He explained, "In the field of biological and medical research the trend is more and more to making precise measurements rather than gross observations. Biology is becoming increasingly quantitative rather than just descriptive. In addition, biological function or malfunction is increasingly being studied at the cellular or even molecular level, and there is a great need for instruments and techniques capable of accurately and rapidly measuring properties and composition of individual cells and cell components.

"In the physical sciences, such as physics and chemistry," he said, "great advances have been made in developing new equipment for the acquisition and analysis of data, and we in H-4 are trying to keep abreast of these developments and are looking for ways in which they can be applied to biological problems. Of course, this effort has grown out of, and is shaped by, the specific needs of the H-4 research program."

The biophysics section, led by Marv Van Dilla, got started in this field several years ago when the group began to get deeply involved in cellular and molecular biology. One of the first problems encountered was that of speeding up and automating the existing manual process for determining cell concentration (cells per cubic centimeter) in cell cultures —that is, a large colony of single cells growing and dividing in a nutrient solution.

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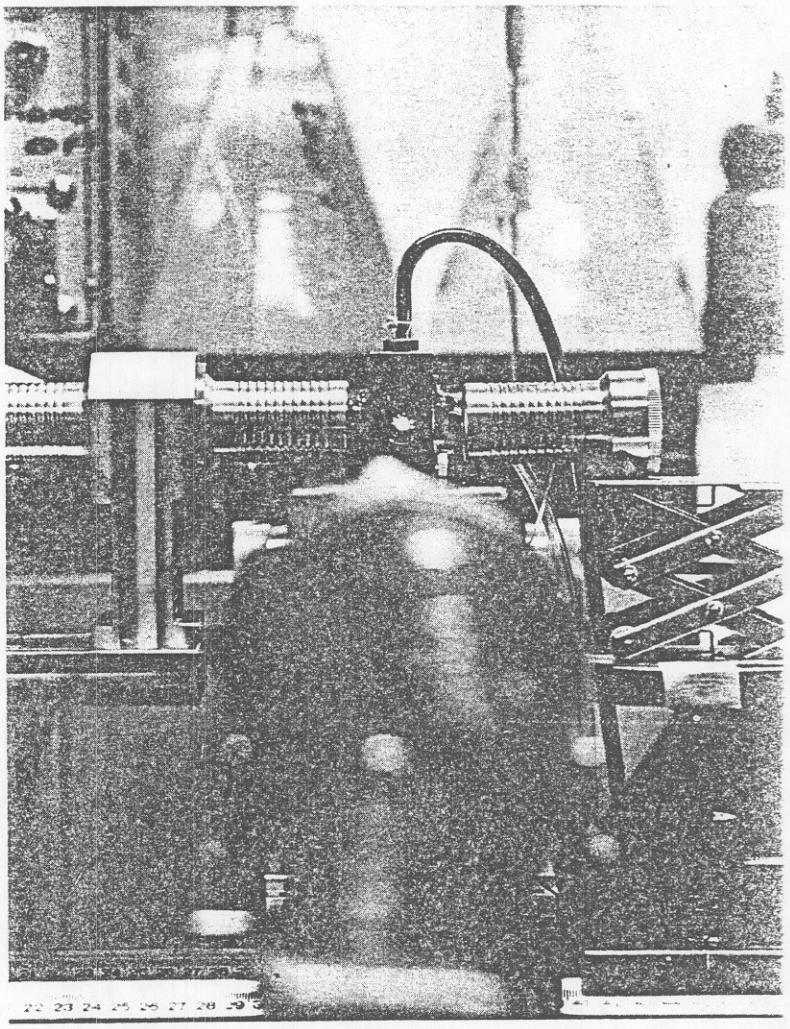

Cells, which have absorbed a fluorescent dye such as acridine orange, flow up through the flow chamber of the fluorescent cell photometer, are illuminated through a chamber window by a beam of blue light coming from the left, and fluoresce. The fluorescent light passes out of the flow chamber through the window facing the camera and into a photomultiplier tube (out of focus in the foreground). FACING PAGE: The blue light from the mercury-vapor lamp passes through windows in the flow chamber and projects this image of the chamber and the tiny stream of cells, here simulated by ink particles for calibration purposes.

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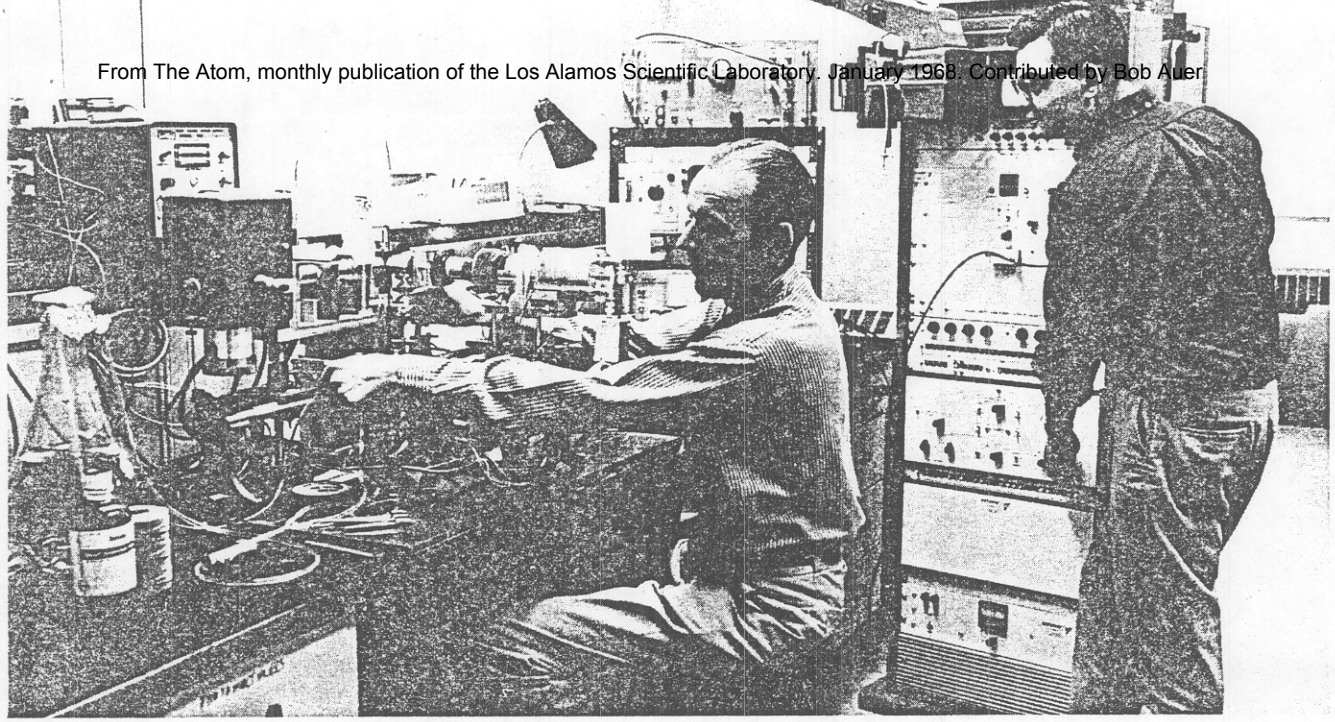

Marv Van Dilla, seated, looks over fluorescent cell photometer H-4 is developing. At left is mercury-vapor lamp producing a beam of blue light to activate fluorescent dye absorbed by the cells. Directly in front of Van Dilla is the photomultiplier tube that detects bursts of light from the cells and converts them into electrical pulses. At right, Paul Mullaney operates the pulse-height analyzer that sorts and totals the signals from the photometer.

Biomedical Research . . .

continued from preceding page

A Coulter cell counter (named after its inventor, Wallace Coulter) with limited capability for spectroscopy was obtained and used for determining cell concentrations. Modifications were made which greatly increased its reliability for use as a cell volume spectrometer for rapid and accurate measurement of the distribution of cell volumes. This spectrometer was then developed into the electronic cell separator.

The electronic cell volume spectrometer consists essentially of a container divided into two sections by a partition. A hole through this partition allows cell culture to flow from one side to the other. The diameter of the hole (100 microns) is approximately ten times the diameter of the cells being counted. (A micron is one millionth of a meter.) Electrodes are immersed in the cell culture in the two sections, and appropriate instrumentation measures the electrical conductivity between the electrodes, which is determined primarily by the conductance of the hole. As a cell passes through the hole, the conductivity is changed by an amount proportional to the volume of the cell, since cells are nonconductive. If the flow rate through the hole is known, the number of cells per cubic centimeter (cc) of cell culture and their individual volumes can be measured.

The signals from the electrode circuit, converted to voltage pulses, are processed by a device called a multichannel pulse-height analyzer. This instrument, widely used in nuclear physics and radiochemistry to analyze various radiation and charged-particle spectra, is a sort of electronic computer that senses the size of the pulses and sorts them into bins. Each bin, or channel, represents a narrow range of sizes such as 9 or 10 microns. The device also totals up the number of pulses in each bin and displays the results as a graph drawn electronically on the face of a cathode ray tube and prints out the data on paper tape. This graph shows the number of cells of each size range that have passed through the hole. The cell volume spectrometer can count as many as 50,000 cells per minute and has been used extensively for studies of cell life cycles and cell populations by the cellular radiobiology section of H-4.

One task accomplished by the spectrometer has been the measurement of the blood cell volume distribution for all the species of laboratory animals in use at H-4.

Work with this instrument led Mack Fulwyler of the biophysics section, currently finishing his work for a Ph.D. at the University of Colorado Medical Center in Denver, to the development of his cell separator. Fulwyler combined the principle of the electronic cell spectrometer with an idea developed at the Stanford Research Institute for a rapid-response, direct-writing recorder. In the Stanford de-

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vice, the conventional pen, with its relatively large inertia, was replaced by a high-speed stream of electrically-charged droplets of ink. These droplets move down between electrically-charged plates and are deflected by the plates to give an ink line recording of the signal impressed on the plates.

In Fulwyler's separator, which has been patented by the AEC in his name, biological cells in suspension in a saline solution first pass through a volume sensor where their size is measured electronically as in the spectrometer. The cell suspension then enters a droplet generator from which it emerges as a tiny jet. A piezoelectric crystal vibrating at about 70,000 cycles per second and coupled to the jet of solution by a plastic acoustic focusing rod causes the jet of cell suspension to be broken up into small, uniform, evenly-spaced droplets. At the instant a droplet pulls away from the jet, it is electrically charged, by means of a time delay circuit connected with the cell-size sensing unit, by an amount proportional to the volume of the cell contained in the droplet.

As the droplet moves between a pair of charged plates, it is deflected into collection vessels by an amount depending on the size of the charge it carries and therefore on the size of the contained cell. Thus, living cells can be sorted into separate vessels according to their size.

One of the chief users of this device, which is currently under commercial development by an instrument company, has been Ernest Anderson of the cellular radiobiology section. This section is studying the life processes of cells and cell systems in order to be able to determine the effects of radiation on a cell in fundamental terms. In order to understand the mechanism of radiation damage it is necessary to understand the nature of the life cycle in a normal, unirradiated cell. It has long been known that cells in the process of division seem to be more sensitive to radiation, and Anderson and his coworkers are studying the process of cell division and its relationship to cell volume and cell age, working with cultures of mammalian cells such as Chinese hamster ovary cells.

Cells in a culture don't all behave in the same way or live the same length of time, so something other than time is needed to determine the biological age of a cell. Cell growth and volume must somehow be linked with cell age, and Anderson and George Bell (T-DOT) have worked on a mathematical model in which cell volume is a function of time, and the probability of cell division is also a function of time. This model has been fairly successful in predicting the behavior of cells in culture, but further experimental data are required.

Anderson wants to determine the probability of cell division as a function of cell volume. This involves separating cells of particular volumes into colonies and measuring the rate of division of each colony of

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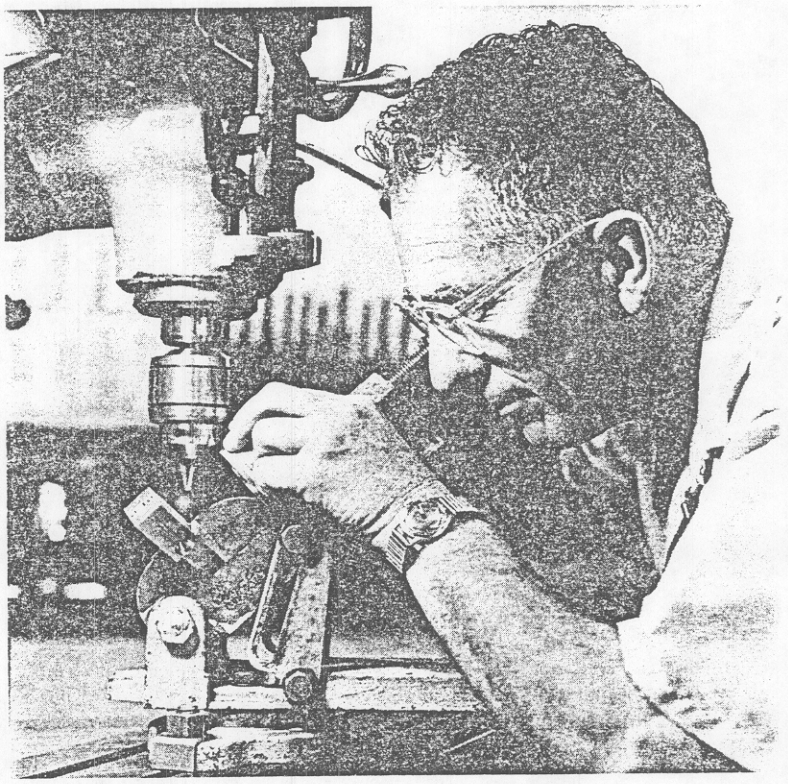

James Coulter, SD-5, of the H-4 branch shop, does some very precise and delicate drilling on a new flow chamber for the fluorescent cell photometer.

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Dick Hiebert, left, and Breck Glascock, P-1, discuss electronics for fluorescent cell photometer with Jim Perrings, H-4.

Biomedical Research . . .

continued from preceding page

cells. This work is complicated by the fact that cell colonies have a critical size—that is, they have to have a density of at least 50,000 cells per cc and a total volume of at least 50cc (2,500,000 cells total) in order to live and reproduce properly. Since the cells being separated may constitute only five per cent of the original cell population, this means that 50,000,000 cells may have to be run through the separator in order to get enough cells of a particular volume to culture.

Present results of this work indicate that after the cells reach a certain volume their probability of undergoing division drops. This doesn't fit existing theories and indicates that much more experimental work and study remain to be done. Anderson is hopeful, however, that this research may be able to provide such information as whether a cell is sensitive to radiation only during division or if the damage only shows up at the time of cell division.

The Fulwyler cell separator has many other scientific applications, one of the most exciting of which is in the field of medical diagnosis. Many diseases—for example, multiple myeloma, mononucleosis and leukemia—affect blood cells, and preliminary work has been done on using the cell separator to investigate the abnormal blood cells caused by such diseases. Results to date hold promise that some day the cell separator may find clinical application in detecting certain blood disorders.

The work on the separator has been followed by a new project. About a year ago the biophysics section began looking at optical methods of detecting cells. They looked at the old and very useful technique of cell staining, in this case with fluorescent dyes, and discovered that they could detect individual cells by the fluorescence of the dye and also by means of light scattered by the cell.

This has led to a new instrument, called a fluorescent cell photometer, with at least as great a potential for research and clinical applications as the cell separator. The photometer measures the amount of dye taken up by individual cells, and since this dye takeup is a function of the biochemical activity of the cell, the photometer is expected to be a powerful tool for the study of such activity.

With the photometer, a fluorescent dye, such as acridine orange, is added to a cell culture and is taken up by the cells. The cell culture is then

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pumped into a water-filled flow chamber with small windows. The chamber and the orifices leading the cell culture solution into and out of it are designed so that the cell culture passes through the chamber in the form of a tiny stream only 75 microns in diameter. The cells line up in this stream and pass through the chamber one at a time at the rate of 10,000 to 100,000 cells per minute.

A beam of blue light, which activates the dye causing it to fluoresce, is focused onto the cell stream through one of the windows in the chamber. The flashes of fluorescent light from the cells pass out of the chamber via another window and are detected by a photomultiplier tube which produces an electrical pulse proportional to the intensity of the light. This pulse is then fed into a multichannel pulse-height analyzer which sorts and adds the pulses and produces a distribution curve showing the number of pulses of each intensity and, therefore, the numbers of cells taking up various amounts of the dye.

The cell fluorescence work has been done using a very intense mercury-vapor lamp, but an argon-ion laser which emits blue light of the proper wavelength (4880 Å) will shortly be put to use as the light source. This laser is not only many times brighter than the mercury-vapor lamp, but also requires only very simple optics rather than the complicated optical system needed with the lamp to collimate and focus the light into the 0.1 millimeter diameter beam needed.

In addition, with this laser it will be possible to focus the light scattered at small angles (less than two degrees) by the cells due to the process of diffraction into a second photomultiplier tube. Since the amount of scattered light is a function of the cell size, this output shows the distribution of cell volumes. Preliminary scattering experiments with a small helium-neon laser confirm the possibility of a dual sensor, measuring two cellular properties simultaneously.

The possibilities of the photometer for cellular research are enormous, since by choosing the appropriate dye a whole range of cellular biochemical functions can be studied.

The acridine orange dye currently being used has the property that it glows green when tied up in the cell nucleus and glows orange when tied up in the cell cytoplasm. There is evidence that in the nucleus it stains DNA and in the cytoplasm RNA. This opens up the possibility of being able to measure directly the DNA-RNA relationships so important to cell division and genetics in general.

A new fluorescent dye has just been received that dyes only the cell membrane, which is very important since everything going into or out of the cell must pass through it. With this dye, the photometer could be used to study cell membrane function. Fluorescent dyes are widely used to study the process of antibody

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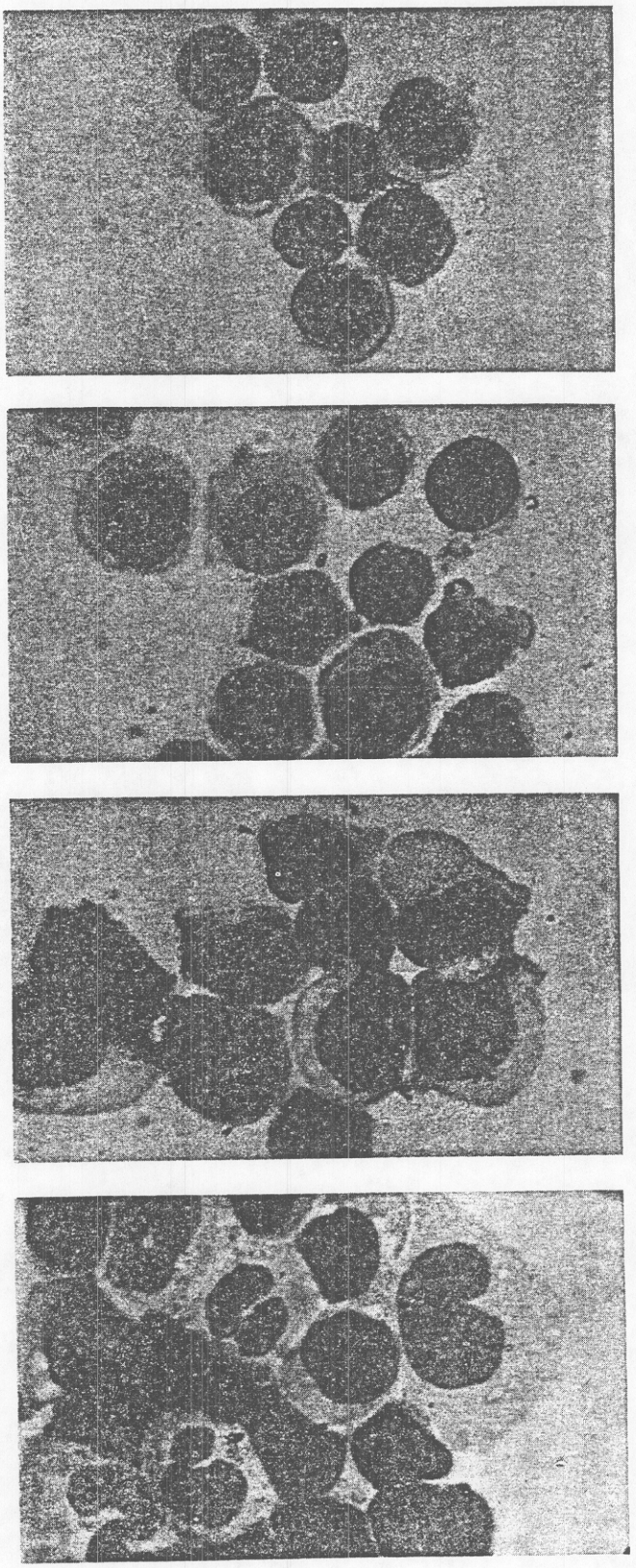

Four different sizes of white blood cells from a patient with infectious mononucleosis were separated by the electronic cell separator.

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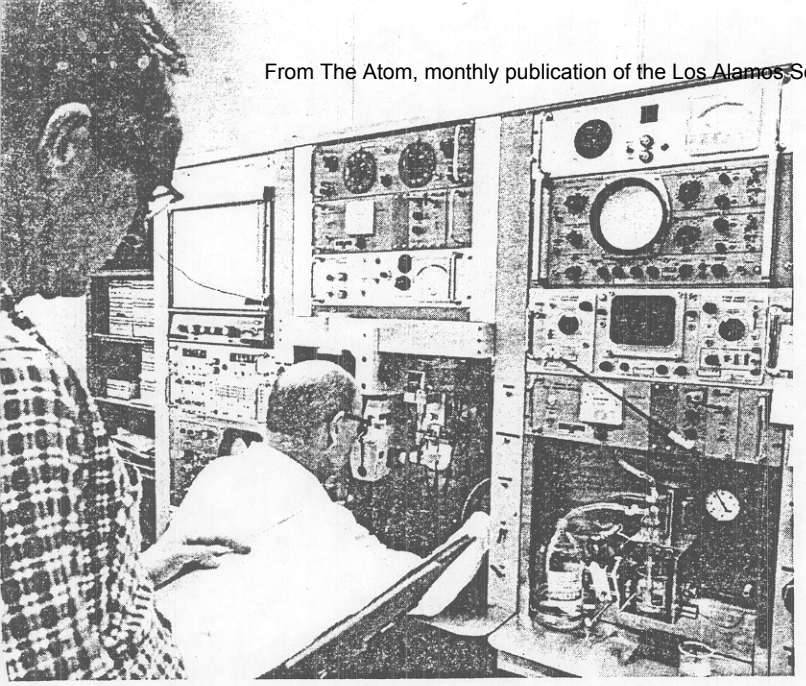

Ernest Anderson looks over data being taken by James Perrings with the cell separator invented by Mack Fulwyler.

Ted Trujillo prepares a sample of blood for processing by the cell separator.

Biomedical Research . . .

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production. The cells producing antibodies—the substances that destroy invading bacteria or foreign protein in our bodies—are thought to be phagocytic cells, but little is known about the site of production or about which cells produce antibodies. Phagocytic cells are freely moving cells, such as the white blood cells, that are produced in the bone marrow, spleen, lymph nodes and liver. By staining bacteria with a fluorescent dye (fluorescein), phagocytic cells producing antibodies could be located and studied since the antibody coats the producing cells and ties it to the bacteria being attacked.

It would also be fairly simple to use the scattered-light, cell-size signal to control the fluorescent light detector so that dye-uptake data would be taken only from cells over and under a certain size. If, for example, small bacteria were mixed up with cells, the fluorescent light signals from the bacteria would be rejected and would not distort the statistics on the cells.

This device, too, clearly has potential medical applications. An obvious one would be the detection of abnormal cells, such as cancer cells, which would not take up a dye in the same manner as a normal cell.

Future plans call for work on new cell separation methods using a fluid switch activated by a signal from the photometer. In the switch—a fluidic bistable developed for use in nonelectric amplifiers and computers—the cell stream would be diverted into one of two channels by small control jets activated by signals from the photometer. In this way cells could be separated on the basis of size, using the signal from the scattered light, or on the basis of biochemical function or malfunction, using the fluorescent light signal, or even some combination of both. By cycling the cells through the system several times or using several photometers and fluidic switches in series, a cell culture could be separated into several groups and would allow cells with certain types of functions to be cultured separately and studied.

Working with Van Dilla on the development of the fluorescent cell photometer are biophysics section members Paul Mullaney (optics), Ted Trujillo (biological technology), Phil Dean (theory and electronics) and John Larkins and Jim Perrings (mechanical and electrical design). In addition, Jim Coulter, SD-5, of the H-4 branch shop, has contributed greatly to the design and fabrication of the instrument; Dick Hiebert, Jim Gallagher and Breck Glascock, all of P-1, have worked on the design and construction of the electronics required; Berlyn Brixner, GMX-9, has provided assistance and advice on the optics; and Frank Harlow of T-3 was consulted concerning fluid dynamics.

This interdisciplinary cooperation, one of the great strengths of an organization such as LASL, will undoubtedly continue in the future to help H-4 to develop even better and more useful equipment for biomedical research.