size and thickness of the emitting region. This requirement is consistent with the constraint of $R < 10^{16}$ cm set by the observation that the core of the x-ray–emitting region is smaller than 1 arc second and the more severe constraint of $< 3 \times 10^{13}$ cm set by the observation of intensity variations on the time scale of 1000 seconds.

While thermal bremsstrahlung from hot plasma is a reasonable interpretation of the x-ray data, detailed models for the x-ray emission from θ^1C or any of the other stars in the Orion Nebula do not exist. Several scenarios for emission from early-type stars with strong stellar winds have been proposed. Our data and other Einstein data on OB stars (*5, 6, 22–24*) constrain some of these wind models. Cold stellar winds seem to be ruled out by the higher temperatures observed. Strong shock heating and the formation of large circumstellar bubbles (*25*) conflict with our observation of a small emission region for θ^1C. Colliding winds from θ^1C and other Trapezium stars (*26*) may not be ruled out (see Fig. 2), but they cannot account for more than a few percent of the total x-ray emission from θ^1C. Hybrid models invoking hot coronas at the base of cold stellar winds (*27*) may eventually yield the right explanation for early-type stars. X-ray emission from late-type stars is also not well understood. In addition to coronal models (*28*), accretion and outflow models (*29, 30*) have been applied to x-ray observations with limited success. The observation of x-ray emission from a wide variety of field stars by the Einstein Observatory (*31, 32*) led Vaiana *et al.* (*31*) and others to suggest that magnetically dominated coronas, similar to that on our sun, must play an important role in all stars. Our observations of the Orion Nebula and other Einstein observations of the Pleiades and the Hyades (*33*) suggest that age and activity, as evidenced by rotation and magnetic fields, also play a part—that is, younger stars with active coronas produce more x-rays. Further analysis of the 29-month Einstein data base should improve our understanding of stellar dynamics and stellar evolution.

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Phagocytosis: Flow Cytometric Quantitation with Fluorescent Microspheres

Abstract. *The phagocytosis of uniform fluorescent latex particles by pulmonary macrophages in the rat was analyzed by flow cytometric methods. The percentage of phagocytic macrophages and the number of particles per cell were determined from cell-size and fluorescence histograms. A comparison of in vivo and in vitro phagocytosis data showed that the percentage of phagocytic lavaged macrophages reflected the availability of instilled particles. With sodium azide used to model phagocytosis inhibition, it was shown that the percentage of phagocytic cells and the number of particles per cell can be determined simultaneously.*

The phagocytosis of inhaled microorganisms and inert particles by pulmonary alveolar macrophages (PAM's) is the primary cellular defense mechanism within the lung (*1*). Any agent that depresses or alters phagocytic action adversely affects the host's susceptibility to disease or destructive lung damage (*2*). Two frequently used criteria of phagocytosis are the percentage of cells having phagocytized one or more particles and the actual number of particles per cell determined microscopically (*3*). The procedure used to measure these values is tedious and time-consuming, and only a small sampling of the total cell population is obtained. Other methods, such as the use of radiolabeled biological material (*4*), are faster but provide no information on the actual proportion of cells that are phagocytic.

We have developed a phagocytosis assay in which cells are incubated with uniform fluorescent latex particles and are analyzed by automated flow cytometric (FCM) cell-analysis methods (*5, 6*). This technique permits rapid quantitation of the percentage of phagocytic cells within the total population and the number of phagocytized spheres per cell. We measured in vivo phagocytosis by instilling 1×10^7 to 2×10^7 green fluorescent latex spheres (1.83 μm in diameter) (*7*) suspended in 0.5 ml of saline into the lungs of anesthetized (15 mg of methohexital sodium, intramuscular) Sprague-Dawley rats. Two hours later the lungs were lavaged four times with 5 ml of saline containing 5 percent newborn bovine serum. Cells were layered over 3 ml of newborn bovine serum and centrifuged for 10 minutes at 360*g*. The cells were pelleted while the nonphagocytized spheres remained at the serum-saline interface. Cells were then washed in saline and fixed for 30 minutes in 70 percent ethanol. Before automated analysis, the cells were centrifuged to remove ethanol

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and resuspended in saline. The percentage of cells phagocytizing one or more spheres was determined microscopically.

To measure phagocytosis in vitro, cells were lavaged from the lungs, centrifuged, and resuspended at a concentration of 10^6 cells per milliliter in alpha minimum essential medium supplemented with 5 percent newborn bovine serum. Then 5×10^7 fluorescent latex spheres in 50 μl of saline were added to each milliliter of cell suspension. Cells and microspheres in polystyrene, nonwettable tubes (Falcon) were maintained in suspension by means of a shaker platform at 37°C. After 30 minutes, cells were separated from nonphagocytized spheres as described above.

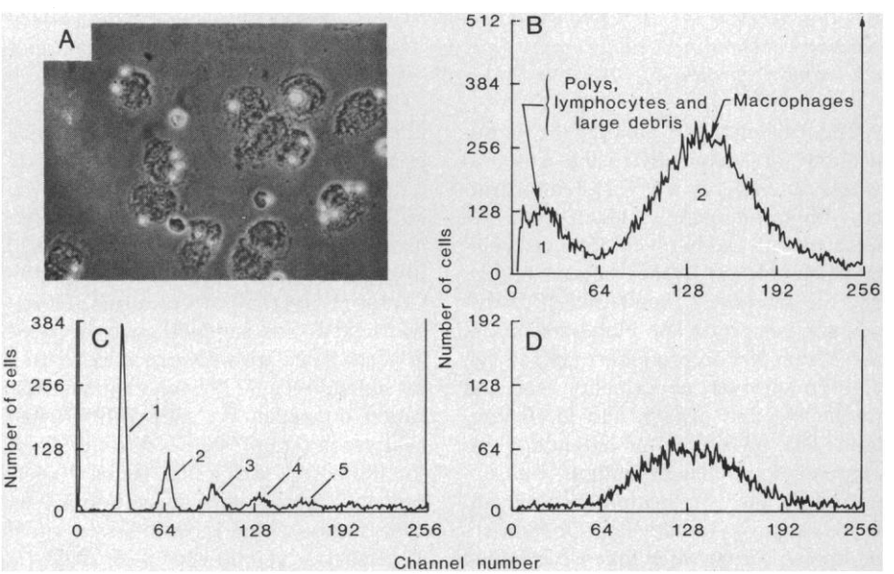
Cells were analyzed for fluorescence (phagocytized spheres) and light scatter (cell size) as they flowed (1000 per second) through a flow cell intersecting the 457.9-nm line from an argon ion laser excitation source (*5*). Optical sensors measured fluorescence and size on a cell-by-cell basis. Signals were displayed as frequency-distribution histograms on an LSI-11 computer data analysis system. Cells were also separated on the basis of fluorescence (number of ingested spheres) and placed on slides for microscopic examination.

The microscopic appearance of cells from lavage samples and the fluorescence and light scatter distributions are illustrated in Fig. 1, A through D. Figure 1B shows the size distribution of lavaged free cells. Region 1 represents polymorphonuclear leukocytes (polys), lymphocytes, and large debris. Macrophages that have or have not phagocytized particles are contained within region 2. Some overlap of populations occurs. Since fluorescent spheres 1.83 μm in diameter are smaller than the cells, they do not appear in the cell-size distribution and thus can be totally discriminated against. Therefore, by requiring fluorescence signals to be in coincidence with light scatter signals from cells, only cells that have phagocytized spheres are contained in the fluorescence distribution. Figure 1C shows the fluorescence distribution of macrophages having phagocytized spheres. Peaks 1 through 5 represent the number of PAM's having phagocytized one, two, three, four, or five spheres, respectively, as verified by the sorting of cells from each peak (see Fig. 2). Macrophages containing more than five spheres are located in channels 180 to 255, with the number in channel 255 represented by a line that goes off scale.

Table 1. Phagocytic properties of rat alveolar macrophages exposed in vitro to sodium azide.

Sodium azide (%)	Percentage of phagocytic cells	Percentage of phagocytic cells containing	
		One to five spheres	More than five spheres
0	91	26	74
0.1	20	69	31
0.3	6	76	24
1.0	7	72	28

Gated analysis methods were used to demonstrate that region 2 of the cell-size distribution (Fig. 1B) contains macrophages that have phagocytized spheres. By displaying only cells associated with fluorescence signals (Fig. 1C), we obtained the size distribution of macrophages containing ingested spheres (Fig. 1D). This distribution illustrates that macrophages are located primarily in region 2. Data from the fluorescence and size distributions can then be used to determine the percentage of cells containing one or more spheres and the percentages of phagocytic cells having ingested one to five spheres or more than five spheres. By dividing the number of PAM's having phagocytized one or more spheres by the number of cells within region 2, we calculated that 25 percent of the cells were phagocytic. These data



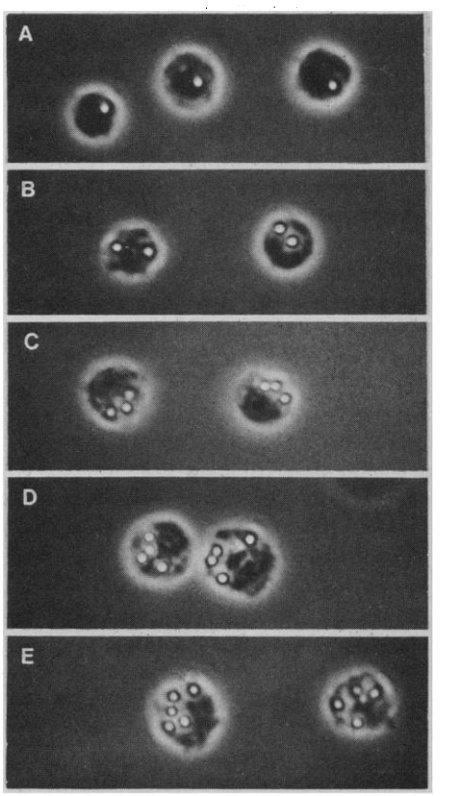


Fig. 1 (left). (A) Photomicrograph (phase-contrast microscope) of pulmonary free cells and spheres lavaged from Sprague-Dawley rat lungs instilled with spheres 1.83 μm in diameter. (B) Cell-size distribution based on small-angle light scatter measurements of lavaged free cells. (C) Fluorescence distribution of macrophage-phagocytized spheres obtained by displaying only fluorescence signals associated with light scatter signals from cells. (D) Cell-size distribution of lavaged macrophages containing phagocytized spheres obtained by displaying only small-angle light scatter signals associated with fluorescence signals. The instrumental reproducibility was greater than 98 percent, as determined by repeated analysis of the same sample. Fig. 2 (right). Photomicrographs of rat pulmonary alveolar macrophages containing spheres 1.83 μm in diameter separated from peaks 1 to 5 of the fluorescence distribution of phagocytized spheres (Fig. 1C). (A) Cells separated from peak 1; (B) cells separated from peak 2; (C) cells separated from peak 3; (D) cells separated from peak 4; and (E) cells separated from peak 5. Cells sorted from peaks 1 through 5 are mononuclear, phagocytic, and have been identified on the basis of their morphological features as macrophages.

were further categorized into the percentages of phagocytic PAM's containing one (27 percent), two (15 percent), three (11 percent), four (8 percent), five (7 percent), and more than five (32 percent) spheres. The average percentage of cells with one or more spheres measured on 41 normal rats was 28.2 ± 8.2 percent.

Since this percentage was lower than anticipated, we designed experiments to determine if all macrophages in the alveolar region were being exposed to spheres and if the spheres were in high enough concentration. Rats were again instilled in vivo with green fluorescent spheres for 2 hours. Samples were washed to remove nonphagocytized spheres and then incubated in vitro for 30 minutes with red fluorescent spheres 1.65 μm in diameter (*7*). Microscopic examination showed that 35 percent of the PAM's had both red and green spheres, more than 60 percent contained only red spheres, and less than 5 percent had no spheres. Thus there were PAM's within the lungs that had not phagocytized green spheres but were capable of phagocytizing red spheres in vitro. These results suggested that the instillation of 1×10^7 to 2×10^7 spheres was insufficient to distribute them to all macrophages within the lungs. To alleviate this problem, we killed the rats, surgically exposed the trachea, opened the thoracic cavity, and instilled 15 ml of green fluorescent spheres (5×10^7 per milliliter) into the lungs hydrostatically (25 cm H_2O) by way of the trachea. After 30 minutes the lungs were lavaged and the percentage of phagocytic cells was quantitated microscopically and by FCM analysis. The results showed that more than 90 percent of the cells were phagocytic.

Finally, to determine if microspheres were being phagocytized or instead were being nonspecifically bound to the cell surface, rat PAM's were exposed in vitro to 0, 0.1, 0.3, and 1.0 percent sodium azide and green fluorescent spheres. Experimental FCM data showed (Table 1) that 91 percent of PAM's that were not exposed to sodium azide had associated spheres, whereas only 6 to 7 percent had spheres associated with them when exposed to 0.3 to 1.0 percent sodium azide. The microscopically determined phagocytoses were within 5 percent of the FCM results. Decreased phagocytosis, induced by sodium azide, was also shown in terms of a reduction in the number of particles per cell. Thus, as determined by FCM, the percentage of PAM's containing one to five spheres increased, whereas the percentage containing more than five spheres decreased. These data show that, as the inhibitor concentration increased, the percentage of phagocytic PAM's decreased and those PAM's that were phagocytic had fewer spheres.

Although Sprague-Dawley rats were used in these studies, results may differ among rat strains and animal species. Since the microspheres contain surface carboxyl groups, they can be coated with opsonins, antibodies, or other chemicals for studying specific receptor-mediated phagocytosis (*8*). This technology has broad application for the rapid and accurate determination of phagocytosis by many cell systems, including the study of the effects of environmental toxicants.

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Serum from Monkeys with Histories of Fetal Wastage Causes Abnormalities in Cultured Rat Embryos

Abstract. *Rat embryos in the mid-head-fold stage were cultured on female monkey serum for 48 hours. On the basis of embryo response, the investigators, with no knowledge of the donors' reproductive histories, correctly identified the serum from two high-risk breeders among 18 rhesus monkeys and the serum from 12 of 14 high-risk breeders among 26 pig-tailed monkeys as teratogenic. Of the embryo response parameters examined, abnormality type and frequency were more closely correlated with donor reproductive histories than embryo protein content or somite number.*

The feasibility of evaluating the teratogenicity of serum by using cultured whole rat embryos was first demonstrated with serum samples taken from rats injected with cadmium chloride or cyclophosphamide (*1*). Depending on dosage and the amount of time between teratogen administration and blood collection, the serum had reproducible effects on embryo survival, abnormality type and frequency, and protein and DNA content. The possibility of extending this approach to studies of humans was supported by the observation that rat embryos can grow and develop for 48 hours on human serum when glucose is added (*2*). Furthermore, serum samples from patients undergoing cancer chemotherapy or taking anticonvulsant medication were found to be teratogenic (*2*). Still, the relevance of these observations to the outcome of actual pregnancies could only be inferred. We now report a relation between the reproductive histories of female monkeys and the teratogenicity of their serum.

Our original objective in using monkeys as serum donors was to introduce primate metabolism as part of a general teratogen-screening procedure involving rat embryo cultures. Serum from 18 female rhesus monkeys (*Macaca mulatta*) from the California Primate Research Center (University of California, Davis) was tested. The samples were prepared in accordance with the procedures used for human serum (*2*), including centrifugation immediately after withdrawal, heat inactivation, sterile filtration, and the addition of antibiotics, water (10 percent by volume), and glucose to a final concentration of 3 mg/ml. Seventy-five rat embryos at mid-head-fold stage (*1*) were cultured in this serum for 48 hours. Serum samples from 16 of the monkeys supported excellent embryo growth and development. Only one of the 75 embryos was considered abnormal, and the mean protein content per embryo was 124 ± 3 μg. Rat embryos were also cultured on serum taken from four of these monkeys at the start of menstruation or on days 8 or 20. All the embryos were morphologically normal and accumulat-

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