Effect of Adriamycin and Analogs on the Nuclear Fluorescence of Propidium Iodide-stained Cells

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ABSTRACT

Adriamycin (ADR) and N-trifluoroacetyladriamycin-14-valerate, respectively, inhibit and enhance the nuclear fluorescence of cells stained with propidium iodide for DNA per cell estimation by flow cytometry. In cells incubated with ADR, the reduction in fluorescence is gradually manifested due to the slow intracellular drug transport. In contrast the effect of N-trifluoroacetyladriamycin-14-valerate on propidium iodide nuclear fluorescence is seen within 5 min of incubation. The effect of ADR on propidium iodide nuclear fluorescence could be detected in vivo even after 24 hr of ADR administration.

INTRODUCTION

FCM² is an important technique for rapid quantitation of cellular DNA content (6, 19). In this system single cells in a suspension are excited with a monochromatic light source (e.g., argon laser beam, 488 nm), and fluorescence of a DNA-dye complex is quantitated at the rate of ~10⁵ cells/min. A number of fluorescent DNA-intercalating drugs (e.g., ethidium bromide, PI, mithramycin) have been used for quantitation of per cell DNA content by FCM (3, 4). The use of these fluorochromes obviates the need for prior fixation and acid hydrolysis of the cells and has led to the extensive use of FCM for rapid cell cycle analysis and determination of aneuploidy. Thus, FCM can provide information on the proliferative status of a cell population within 15 min of sample retrieval.

PI, an analog of ethidium bromide, apparently binds to double-stranded RNA and DNA, primarily by intercalation, and the quantum efficiency of the bound dye ethidium bromide is enhanced 25-fold over that of the unbound dye (3). We have described earlier a PI-hypotonic citrate method for per cell DNA quantitation by FCM (9). This method is rapid and reliable and can yield DNA distribution histograms similar to or better than those obtained by other methods (7, 14). In a recent report Callis and Hoehn (2) have demonstrated the use of this staining method for rapid diagnosis of aneuploidy in peripheral blood cells. In their study chromosomal variations as small as the presence of an extra X (XXY) were rapidly identified.

With proper instrumentation and precautions (e.g., uniform flow rate and proper alignment of the flow channel), it is reasonably possible to obtain highly reproducible quantitation of per cell DNA content by FCM. However, it is conceivable that exposure of cells to other DNA-intercalating drugs (e.g., in cancer chemotherapy) may alter binding of PI to DNA and thus yield erroneous results. This report describes our observations on the effect of a number of cancer chemotherapeutic agents (e.g., ADR, AD-32, ACT-D, methotrexate, vinblastine, vincristine), chelating agents (EDTA, sodium citrate), and other agents (e.g., heparin, trypsin, Tween 80) on the fluorescence of cells stained with PI. This study also shows the potential of this method for detecting the presence of a DNA-intercalating agent (e.g., ADR) in the nuclei of single cells.

MATERIALS AND METHODS

Log-phase cultures of CCRF-CEM human lymphoblasts were grown in suspension cultures and nourished with Eagle's minimal essential medium (for spinner cultures), supplemented with 10% fetal calf serum and the antibiotics, penicillin and streptomycin. Human peripheral blood was collected from healthy donors in blood collection tubes containing potassium EDTA as an anticoagulant. Mononuclear cells were separated by centrifugation over a Ficoll-Hypaque gradient, and washed twice in HBSS. Single-cell suspensions were prepared from spleen and liver of DBA/2 mice by mincing and filtering of the tissues through glass wool and 70 μm monofilament nylon cloth (Small Parts, Inc., Miami, Fla.). Male C57BL × DBA/2 F₂ mice were given i.p. injections of 10⁷ P388 mouse ascites cells maintained in DBA/2 mice by weekly transplantation. Twenty-seven tumor-bearing mice were divided at random in 3 groups of 9 each. Animals in Groups 1 and 2 were given i.p. injections of ADR, 1 or 4 mg/kg body weight (average weight, 20 g) in 0.1 ml of diluent (HBSS). The remaining 9 animals served as controls, and 2 of these were given injections of 2 and 8% Tween 80 diluted in HBSS, respectively. Animals (3 from each ADR group) were sacrificed after 1, 6, and 24 hr of drug administration. Tumor cells from 1 ml of ascites were retrieved by centrifugation and resuspended in 2 to 5 ml of PI solution to give a final cell concentration of 1 to 2 x 10⁷ cells/ml. Ascites cells from control animals were similarly treated.

PI was purchased from Calbiochem, San Diego, Calif., and staining solutions (henceforth referred to as PI solution) were made by dissolving 5 mg of the dye in 100 ml of GDW. ADR-HCL (NSC 123127) and AD-32 (NSC 246131)
were made as 1-mg/ml stock solutions in 10% Tween 80 (ADR and AD-32) or in HBSS (ADR) and further diluted in HBSS or GDW immediately before use. ACT-D (Cosmegen; Merck, Sharp and Dohme, West Point, Pa.), vincristine and vincristine sulfate (Eli Lilly and Co., Indianapolis, Ind.), and methotrexate stock solutions were made in HBSS. In all staining protocols an approximate cell concentration of 1 to 2 \times 10^6\text{cells/ml} was maintained unless stated otherwise.

**Fluorescence Microscopy.** Cells stained with ADR, AD-32, or PI were scanned for their fluorescence emission spectra under a Zeiss fluorescence microscope fitted with a mercury light source (HBO-200), Zeiss III RS epiluminator, and a Nanometrics ultrascotrophotometer (Nanometrics Inc., Irvine, Calif.). Filters used were BG-38 and BG-12 (peak transmittance, 400 nm) for excitation and barrier filter no. 53 (transmittance, 530 nm) for emission.

Fluorescence of single cells (or nuclei after hypotonic disruption of the cell membrane) stained with ADR, AD-32, and/or PI was measured either with a Zeiss 03 microscope photometer or under an Olympus BH/RFL quantitative microscope fitted with an epiluminator for fluorescence. In the Olympus unit a high-pressure mercury light source (HBO-100) was used with the following filter combination: exciter, BG-12; dichoric mirror, DM-445; and barrier filter transmitting above 590 nm. All measurements were made under a \times 40 oil immersion (nonfluorescing) Olympus objective (N.A. 1.0) with a 30-\mu m illuminating spot.

Excitation and emission spectra of ADR, AD-32, and PI solutions (with or without calf thymus DNA (1 mg/ml) were obtained on a Hitachi Perkin-Elmer MFP-2A fluorescence spectrophotometer. For excitation spectra the emission monochromator was set at 580 nm for ADR, 590 nm for AD-32, and 590 nm for PI. For emission spectra the excitation monochromator was set at 488 nm.

**Flow Analysis.** Samples were analyzed on a Model 4801, Cytofluorograf (Bio/Physics Systems Inc., Mahopac, N.Y.) fitted with an improved flow channel, sheath water flow, and externally stabilized photomultiplier power supply. Details of our instrumentation have been reported previously (14). Briefly, in this flow system, the fluorescence of cells individually excited by an argon laser beam (488 nm) is quantitated and scaled in a pulse height distribution analyzer. The abscissae of the histograms generated are divided into 512 channels of increasing linear value, and the number of cells in each channel is recorded on the ordinate. With our improved flow system, we are routinely obtaining a CV of 5 to 7% for the G, peak of PI-stained human lymphoblasts.

Samples were incubated at an approximate cell concentration of 10^6\text{cells/ml}, and for FCM analysis the flow rate was maintained between 1 and 2 \times 10^6\text{cells/sec}. Under these conditions the CV of the G, peak in PI-stained peripheral blood mononuclear cells was 6.5%. In 10 consecutive runs of 40,000 cells each, the mean G, peak channel number was 124.72 \pm 1.173 (S.D.). In subsequent runs laser power was increased to coincide the peak of PI-stained mononuclear human cells with Channel 200 \pm 2.2 of the pulse height distribution analyzer. P388 tumor cells in vivo were analyzed with the G, peak at Channel 150 \pm 1.9.

To confirm and compare these data obtained on the Bio/Physics Cytofluorograf and at the suggestion of the reviewers, we have recently used a Coulter Electronics TPS I cell sorter fitted with a 4-watt Spectraphysics Model 164 laser to analyze ADR-, AD-32-, and PI-stained cells. DNA distribution histograms of PI-stained lymphoblasts analyzed in this instrument at a laser power setting of 1 watt gave a CV of 3 to 4%. In 10 consecutive runs of PI-stained lymphoblasts (100,000 each), the peak channel of G, DNA content did not deviate by more than \pm 1 channel.

**RESULTS**

**Excitation and Emission Characteristics**

Excitation spectra in Chart 1A show that the fluorescence of ADR, AD-32, and PI is maximally excited between 460 and 480 nm. Emission spectra for ADR and AD-32 (Chart 1B) are similar with peaks at 550 and 590 nm. AD-32 in equimolar concentration is less fluorescent than is ADR. PI (unbound) has an emission peak at 590 nm, but the overall fluorescence intensity of unbound PI (excitation at 488 nm) is less than that of ADR or AD-32. Addition of calf thymus DNA, 1 mg/ml, to ADR, AD-32, or PI solution has significant effects on the fluorescence intensity. As seen in Chart 1B, addition of calf thymus DNA to ADR resulted in pronounced fluorescence quenching, whereas no effect was seen on the fluorescence of AD-32 (curve not shown). In contrast addition of calf thymus DNA to PI solutions enhanced the fluorescence approximately 20-fold.\footnote{These observations are similar to those of Dr. Harry A. Crissman, who was generous enough to send us copies of his unpublished spectra on PI.}

For measurement of the emission spectra of stained cells, PI and ADR dilutions were made in hypotonic GDW to facilitate diffusion across the cell membrane and nuclear binding. In view of the earlier reported (13) rapid appearance and localization of AD-32 fluorescence in cytoplasm (no detectable fluorescence in nuclei), AD-32 solutions were also made in isotonic HBSS. Cells stained with PI after hypotonic disruption of the cell membrane have bright nuclear fluorescence with a broad emission peak at 610 nm. Both ADR- and AD-32-stained cells have major emission peaks at 560 nm and a smaller peak at 540 nm (Chart 1C). However, PI-stained single nuclei were approximately 20-fold more fluorescent than are nuclei stained with ADR or cells stained with AD-32.

**Transport and Fluorescence of ADR, AD-32, and PI**

CCRF-CEM and P388 cells incubated in isotonic solutions of the 3 fluorochromes confirmed the earlier reported data on the transport and localization of these agents (9, 13, 16). ADR (1 to 10 \mu g/ml) slowly enters the cells, and nuclear and cytoplasmic fluorescence of ADR can be gradually detected, reaching a peak after the second hr of incubation. Nuclei and chromatin are brightly fluorescent with faint red cytoplasmic fluorescence. Cells incubated in hypotonic solutions of ADR (GDW) show rapid appearance of fluorescence and its localization in nuclei and in chromosomes. Fluorescence of cells stained with ADR (1 to 10 \mu g/ml in GDW) and examined in the Cytofluorograf can hardly be detected even with maximum available laser power and amplification. Fluorescence of most of the population is

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**ADR and PI Nuclear Fluorescence**
Effect of Diluent on Fluorescence

Solutions of 10% Tween 80 had no measurable fluorescence when excited at 488 nm in the spectrofluorometer. Addition of 0.1 to 1% Tween 80 to PI staining solution had no effect on the amount of PI nuclear fluorescence in CCRF-CEM cells. Similarly, in animals given injections of 0.1 ml of 2 or 8% Tween 80, the PI nuclear fluorescence of P388 ascites cells was similar to that of cells from animals given injections of HBSS.

Photometric Measurement of Fluorescence

Although the Olympus BH/RFL and the Zeiss fluorescence use a similar mode of epillumination and filter combinations, the 2 systems do differ in an important detail. In the Zeiss microscope-photometer, fluorescence of individual cells was measured by moving the cell to the central photometer measuring spot. In the Olympus BH/RFL microscope, a cell was located under tungsten lamp illumination and then illuminated with a narrow 30-μm spot of excitation from the mercury source. Because PI nuclear fluorescence does not appreciably decay on excitation from a mercury source, no major differences were noted in measurements made with the 2 different methods of illumination. Reduction in the amount of PI nuclear fluorescence was seen in all types of cells (e.g., human peripheral blood lymphocytes, CCRF-CEM lymphoblasts, liver, and spleen cells) exposed to hypotonic solutions of ADR. Reduction in fluorescence was proportional to the ADR concentration as well as to the length of exposure (in cells exposed to isotonic solutions of ADR). In contrast AD-32, which does not show nuclear fluorescence, slightly enhanced (10%) the fluorescence of PI-stained nuclei. Because of its rapid transport, toxicity of AD-32 solutions had no effect on the PI nuclear fluorescence. These observations are in contrast to those on ADR dissolved in isotonic solution in which reduction in PI fluorescence was gradually manifested and reached a maximum only after 3 hr of incubation at 37° (see next paragraph).

Data in Table 1 show the fluorescence intensity values of cells (liver and spleen) resuspended in PI solution that contains various concentrations (1 to 10 and 100 μg/ml) of ADR, AD-32, or ACT-D. In both liver (2c, 4c DNA content) and spleen cells, a maximum reduction (44 to 46% of control) in PI nuclear fluorescence was seen after exposure to ADR (100 μg/ml). Exposure to 1 and 10 μg/ml of ADR caused a corresponding reduction in PI fluorescence. In spleen nuclei exposed to PI that contain ADR (1 μg/ml), we could not detect any reduction in fluorescence although liver nuclei similarly treated had reduced fluorescence (85 to 86%). In contrast the effects of AD-32 on PI nuclear fluorescence were less significant. Enhancement of PI nu-

4 Fluorescence of PI-stained nuclei illuminated with a HBO-200 mercury lamp (excitation filter BG-12, emission filter 590), under a Zeiss microscope was measured with a Nanometrics ultraviolet photometer and the Zeiss photometer. Fluorescence values recorded after 1 min of constant illumination were approximately 2% lower than were those recorded at the start. Most of this loss happened during the initial 30 sec, and the values remained remarkably constant (less than 1% decay) on further illumination for up to 10 min. We presume that this indicates resistance of PI nuclear fluorescence to photodecay on illumination from a HBO-200 mercury source and the appropriate filter combination.
clear fluorescence (112% of control) was seen in spleen cells exposed to AD-32 (100 µg/ml); whereas liver cells showed a small reduction in the fluorescence. In cells exposed to ACT-D, a reduction in PI nuclear fluorescence, that in turn was related to ADR concentration and the length of incubation. In one set of experiments, we compared the above observations with those for human peripheral blood lymphocytes incubated with either ADR (5 to 10 µg/ml) mixed in the PI solution (hypotonic) or with ADR (5 to 10 µg/ml) dissolved in GDW (isotonic) prior to staining with PI. Exposure of cells to hypotonic media resulted in cytoplasmic lysis and the rapid entry of ADR and PI into the nuclei. Histograms in Chart 2 show the PI nuclear fluorescence peaks from this series of experiments. As expected cells incubated in hypotonic ADR (5 µg/ml) Chart 2, (Histogram A) had a lower amount of PI nuclear fluorescence (Channel 135, -32%) than did the cells stained with PI after incubation in isotonic ADR solution (Histogram B) for 2.5 hr (Channel 177, -11%) or cells stained with PI alone (Histogram C, Channel 199, 100%. Similarly, Histogram E (Chart 2) shows that exposure to higher ADR concentration (10 µg/ml, HBSS) further reduced the amount of PI nuclear fluorescence and that the peak shifted from a control (PI alone) value of 199 to 167 (-16%). Cells incubated in hypotonic ADR solutions (GDW) for 15 min and subsequently resuspended in PI solution showed a further reduction (45.7%) in the amount of PI nuclear fluorescence (peak at Channel 108, Histogram D).  

**ADR in Vitro.** CCRF-CEM lymphoblasts and peripheral blood lymphocytes incubated with ADR (0.1 µg/ml) (isotonic), for up to 6 hr and subsequently stained with PI showed no apparent reduction in the amount of PI nuclear fluorescence. However, cells incubated with ADR concentrations higher than 0.1 µg/ml had a reduced amount of PI nuclear fluorescence, that in turn was related to ADR concentration and the length of incubation. In one set of experiments, we compared the above observations with those for human peripheral blood lymphocytes incubated with either ADR (5 to 10 µg/ml) mixed in the PI solution (hypotonic) or with ADR (5 to 10 µg/ml) dissolved in GDW or HBSS (isotonic) prior to staining with PI. Exposure of cells to hypotonic media resulted in cytoplasmic lysis and the rapid entry of ADR and PI into the nuclei. Histograms in Chart 2 show the PI nuclear fluorescence peaks from this series of experiments. As expected cells incubated in hypotonic ADR (5 µg/ml) Chart 2, (Histogram A) had a lower amount of PI nuclear fluorescence (Channel 135, -32%) than did the cells stained with PI after incubation in isotonic ADR solution (Histogram B) for 2.5 hr (Channel 177, -11%) or cells stained with PI alone (Histogram C, Channel 199, 100%. Similarly, Histogram E (Chart 2) shows that exposure to higher ADR concentration (10 µg/ml, HBSS) further reduced the amount of PI nuclear fluorescence and that the peak shifted from a control (PI alone) value of 199 to 167 (-16%). Cells incubated in hypotonic ADR solutions (GDW) for 15 min and subsequently resuspended in PI solution showed a further reduction (45.7%) in the amount of PI nuclear fluorescence (peak at Channel 108, Histogram D).  

**AD-32 in Vitro.** In contrast to the reduced amount of PI nuclear fluorescence in cells incubated with ADR as described above, cells incubated with the ADR analog AD-32 showed an enhancement in the amount of PI nuclear fluorescence. Unlike ADR the effect of AD-32 on PI nuclear fluorescence was seen within 5 min of incubation in the isotonic drug-containing medium.  

Chart 3 shows PI nuclear fluorescence peaks of human peripheral blood lymphocytes incubated in PI staining solution that contained AD-32, 10 µg/ml (A) (-41%) or 5 µg/ml (B) (-28%), or stained with PI alone (C) (100%). These results are similar to those described previously for human lymphoblasts and P388 tumor cells. However, cells incubated with AD-32 (5 µg/ml) and subsequently stained with PI showed enhancement (+11%) in the amount of PI nuclear fluorescence (channel peak at 219) as shown in Chart 3, Histogram D.  

**Coulter Electronics TPS Cell Sorter.** Mouse tumor ascites (P388) and human peripheral blood lymphocytes exposed to various ADR concentrations, dissolved in PI solution, and analyzed on the Coulter Electronics TPS cell sorter confirmed the data obtained on the Bio/Physics Cytofluorograf and described above. In one set of experiments, P388 cells incubated for 15 min in PI solution that contained ADR (1, 10, and 100 µg/ml) showed, respectively, 3.8, 32.6, and 48% reduction in the amount of PI nuclear fluorescence (as measured by the shift of G1 peak). Similarly, P388 cells incubated for 30 min in various ADR concentrations (GDW) followed by PI staining for 15 min had a more pronounced reduction in PI nuclear fluorescence. Thus, PI nuclear fluorescence in cells exposed to ADR (10 and 100 µg/ml) was 68 and 41% of control, respectively. In contrast to ADR cells exposed to AD-32 either concurrently with PI or first to AD-32 followed by PI showed an enhancement of PI nuclear fluorescence. In one set of experiments analyzed on the TPS cell sorter, we recorded...
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Relative Amount of Fluorescence

Chart 2. PI nuclear fluorescence peaks of mononuclear cells from human peripheral blood incubated with ADR (5 µg/ml) and PI (A, Channel 135, -32%); ADR (5 µg/ml) in isotonic HBSS for 2.5 hr followed by staining with PI (B, Channel 177, -11%); and in PI alone (C and F, Channel 199, 100%). In D and E, cells were incubated in ADR (10 µg/ml) dissolved in GDW (D, Channel 108, -45%) or in isotonic HBSS (E, Channel 167, -16%).

15, 19.6, and 27.4% increase in the fluorescence of P388 cells exposed to AD-32 (1, 10, and 100 µg/ml respectively). These observations confirm earlier data obtained on the Bio/Physics Cytofluorograf.

ADR in Vivo. In tumor-bearing animals given i.p. injections of various concentrations of ADR, we noticed a similar reduction in the amount of PI nuclear fluorescence (Table 2). In this series of experiments, P388 leukemia-bearing mice were given injections of ADR (1 and 4 mg/kg body weight), and ascites were removed at 1, 6, and 24 hr after drug injection. In samples retrieved after 1 hr, a small decrease in the amount of PI nuclear fluorescence (~3%) was noted. In samples analyzed after 6 hr of drug administration, there was a reduction (12%) in the amount of fluorescence, and the peak had shifted from a control (PI alone) value of 150 to 134.4 (1 mg/kg) and 131.8 (4 mg/kg). A similar reduction in PI nuclear fluorescence was evident in tumor cells removed from animals after 24 hr of single ADR injection (Table 2). Comparable results were obtained in cells analyzed in the Coulter Electronics TPS cell sorter (data not included here).

ACT-D, Vinblastine, Vincristine and Methotrexate in Vitro. CCRF-CEM cells incubated in various isotonic (HBSS) or hypotonic (GDW) concentrations of these drugs (1 to 10 µg/ml) for 1 to 4 hr and subsequently resuspended in PI solution did not show any shifting of the G1 peak in the Cytofluorograf.

Chelating and Other Agents. EDTA (Na2, Na+, and K3), sodium citrate, and 1, 10-o-phenanthroline (1 to 10 µg/ml) dissolved in HBSS or GDW had no detectable effect on PI nuclear fluorescence of human peripheral blood cells and CCRF-CEM lymphocytes incubated for 1 to 4 hr prior to staining with PI. Similarly, no effect was seen in cells incubated directly in a mixture of these agents with PI solution. In contrast cells incubated with heparin (sodium heparin, 14 USP units/ml) and subsequently stained with PI did not always yield reproducible DNA distribution histograms although the channel position of the G1 peak was similar to that of cells stained with PI alone. We have occasionally experienced similar problems with tissue culture cells removed from the substrate by routine trypsinization prior to their staining with PI. Microscopic examination of these preparations has ruled out the possibility that cytoplasmic remnants (containing RNA) were responsible for this effect.

DISCUSSION

ADR, a DNA-intercalating antibiotic, inhibits RNA and DNA synthesis and blocks cell cycle traverse in G2 (5, 11,
Talent of rapid FCM analysis for detecting the presence of a
differences in DMA configuration and availability of binding
sites remains to be elucidated.

Experiments in this report (see also Ref. 10) show the po
of ADR and AD-32 to the slower appearance of
ADR fluorescence. We also showed that the fluorescence
of AD-32 is localized in the cytoplasm and does not appear
in nuclei or chromosomes (15). AD-32 resembles ADR in
causing chromosomal damage, in inhibiting RNA and DNA
synthesis, and in causing a block of the cell cycle traverse
in G2 (12).

This study confirms the rapid intracellular appearance of
AD-32 and furthermore shows differences in the effect of
ADR and AD-32 on the fluorescence of PI-stained nuclei.
Thus, ADR causes reduction in the amount of PI nuclear
fluorescence in contrast to AD-32, which enhances the
fluorescence of PI-stained nuclei. These studies indicate:
(a) the probable presence of AD-32 in nuclei; and (b) a
possible difference between AD-32 and ADR in their mode
of binding to nuclei. These observations are interesting in
view of our earlier observations on the lack of any visible
detectable fluorescence in nuclei or chromosomes of cells
incubated with AD-32 (13) and the reported lack of its
binding to calf thymus DNA (17).

Experiments in this report (see also Ref. 10) show the poten
tial of rapid FCM analysis for detecting the presence of a
drug (e.g., ADR or AD-32) in nuclei from various tissues by
measuring the effect of the drug on PI binding. As shown in
this study, it was possible to detect the interference on ADR
with PI nuclear fluorescence in tumor cells removed 24 hr
after in vivo drug injection. Examination of these nuclei
under UV microscopy did not reveal any nuclear fluores
cence; however, the FCM could detect the presence of the
bound drug. This technique could be used to detect the
presence of the bound ADR in various animal tissues, and
studies are underway to analyze this method for scanning
liver, bone marrow, and tumor cells in animals given injec
tions of various anthracyclines (A. Krishan, unpublished
observations).

This study also shows that the interference of ADR with
PI nuclear fluorescence varies from tissue to tissue. Whether this is due to differential binding of ADR or to
differences in DNA configuration and availability of binding
sites remains to be elucidated.

This study indicates the need for caution in the use of
rapid FCM analysis for aneuploidy determination. It is conceivable that, besides ADR, many other chemothera
peutic agents may alter the PI nuclear fluorescence and
give false results.6

ACKNOWLEDGMENTS
We owe our thanks to Harry A. Crissman of Los Alamos Scientific Laboratory, Los Alamos, N. M., for sharing with us his unpublished data on PI fluorescence. To Peter T. Monachese of the Olympus Corporation of America, New York; we are thankful for the loan of the Olympus BH/RFL quantitative microscope. Dr. Awtar Krishan owes his gratitude to Dr. Emil Frei, III, Director of the Sidney Farber Cancer Institute, for the loan of equipment needed for the completion of this project.

REFERENCES
1. Alabaster, O., Tannenbaum, E., Habbersett, M. C., Magrath, I., and
Her man, C. Drug-Induced Changes in DNA Fluorescence Intensity
Detected by Flow Microfluorometry and Their Implications for Analysis of
2. Callis, J., and Hoehn, H. Flow-Fluorometric Diagnosis of Euploid and
Aneuploid Human Lymphocytes. Am. J. Human Genet., 28: 577-584,
1976.
ment of DNA, Protein and Cell Volume in Single Cells from Large
A New Antibiotic with Antitumor Activity. Cancer Chemotherapy Rept.,
6. Dittrich, W., and Gohde, W. Impulsfluorometrie bei Einzelzellen in
Analysis of Cell Cycle Distributions Using Propidium Iodide. Properties
of the Method and Mathematical Analysis of the Data. J. Cell Biol., 71:
8. Israel, M., and Frei, E., III. N-Trifluoroacetyladriamycin-14-
Valerate, an Analogue with Greater Experimental Antitumor Activity and
9. Krishan, A. Rapid Flow Cytfluorometric Analysis of Mammalian Cell
12. Krishan, A., Frei, E., III, and Paika, K. Studies on the Biological Action of
Adriamycin Analog, N-Trifluoroacetyladriamycin (AD-32). Proc. Am. As-
13. Krishan, A., Israel, M., and Frei, E., III. Differences in
Cellular Uptake and Cytosfluorescence of Adriamycin and N-Trifluoroac
14. Krishan, A., Pitman, S. W., Tattersall, M. H. N., Paika, K. D., Smith, D.
C., and Frei, E., III. Flow Microfluorometric Patterns of Human Bone

6 In a recent report Alabaster et al. (1) have demonstrated the interference
of cyclophosphamide with fluorescent binding of mitomycin in vivo
to nuclei of L1210 cells.
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