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Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine

(DNA synthesis/cell cycle/1-β-D-arabinofuranosylcytosine/monoclonal antibody)

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ABSTRACT **We have developed a procedure for simultaneous flow cytometric measurement of cellular DNA content and amount of BrdUrd incorporated into cellular DNA. Propidium iodide was used as a fluorescent probe for total cellular DNA and a monoclonal antibody against BrdUrd was used as a probe for BrdUrd incorporated into DNA. Fluorescein-labeled goat anti-mouse antibody was used to fluorescently label the bound anti-BrdUrd probe. Bivariate DNA/BrdUrd distributions measured for Chinese hamster ovary cells labeled for 30 min with BrdUrd clearly show the G_1- and G_2M-phase cells to have low BrdUrd-linked fluorescence and the S-phase cells to have high BrdUrd-linked fluorescence. Cell cycle traverse rates were estimated for Chinese hamster ovary cells from bivariate distributions measured for samples taken periodically after pulse labeling with BrdUrd. Bivariate DNA/BrdUrd distributions were also applied in the analysis of the response of C3H murine bone marrow cells to treatment *in vivo* with 1-β-D-arabinofuranosylcytosine (araC). Bivariate distributions were measured for bone marrow cells taken from mice that were pulse labeled with BrdUrd at various times after treatment with araC. The resulting DNA/BrdUrd sequences show the kinetics of recovery from araC and allow discrimination of the araC sterilized cells.**

A broad range of biological and biomedical investigations depends on the ability to distinguish DNA synthesizing cells. Oncologists, for example, have devoted substantial effort to establishing correlations between the frequency of DNA synthesizing human tumor cells and the treatment prognosis (1). Effort has also been devoted to improvement of anticancer therapy with S-phase specific agents by treating when the experimentally determined frequency of tumor cells in S phase is maximal (2). In these studies, S-phase cells are usually assumed to be those that appear labeled in autoradiographs prepared immediately after pulse labeling with [^{3}H]dThd or those with S-phase DNA content in DNA distributions measured flow cytometrically. Cytokineticists have relied heavily on measurements of the frequency of DNA synthesizing cells to determine the cell cycle traverse characteristics of normal and malignant cells. The classical "fraction of labeled mitosis" procedure (3), for example, depends on assessment of the frequency of mitotic cells that appear radioactively labeled in autoradiographs of samples taken periodically after labeling with [^{3}H]dThd. Studies of the cell cycle traverse characteristics of drug-treated cell populations (4) typically require measurement of the amount of [^{3}H]dThd incorporated by cells in S phase (e.g., by liquid scintillation spectrometry) or determination of the fraction of cells with S-phase DNA content (e.g., by DNA distribution analysis), or both. Studies of mutagen-induced genetic damage that use unscheduled DNA synthesis as an index of damage also rely on the detection of low levels of incorporation of [^{3}H]dThd (5).

These broad-ranging biomedical studies are often limited by the measurement techniques. For example, autoradiographic determination of the fraction of cells incorporating radioactive DNA precursors like [^{3}H]dThd is limited by the labor-intensive nature of the measurements and by the subjectivity associated with discrimination between unlabeled and weakly labeled cells (6). Determination of the amount of radioactive DNA precursor incorporation by liquid scintillation is also suboptimal because no information is available about the distribution of radioactivity incorporation among the cells of the population. Finally, interpretation of DNA distributions measured by flow cytometry is difficult because no information is available about the absolute rate of DNA synthesis (7). Thus, a population proliferating with twice the rate of another might have the same DNA distribution if the two populations spend the same fractional times in the G_1, S, and G_2M phases of the cell cycle. Furthermore, discrimination between actively synthesizing and quiescent cells with S-phase DNA content is impossible.

We now report the development of a powerful, yet simple, flow cytometric procedure that promises to decrease or remove many of the limitations inherent in earlier methods for study of cell proliferation. Our procedure is based on the simultaneous flow cytometric measurement of cellular DNA content and amount of incorporated BrdUrd. For these measurements, propidium iodide (PrdI) is used as the probe for total DNA content and a monoclonal antibody against BrdUrd (8) is used as the probe for BrdUrd incorporated into DNA. The flow cytometric measurements are arranged to form bivariate distributions of DNA content vs. BrdUrd content. These DNA/BrdUrd distributions readily show the relative amounts of BrdUrd incorporated into the DNA of cells whose DNA contents place them in the G_1, S, and G_2M phases of the cell cycle. This procedure is sufficiently sensitive to allow quantification of very low levels of BrdUrd incorporation (e.g., the incorporation that occurs during a 3-min labeling period). Further, it allows discrimination between DNA synthesizing and nonsynthesizing cells with S-phase DNA content. It is rapid, and it is applicable *in vitro* and *in vivo*. We demonstrate the utility of the technique by applying it to the cytokinetic analysis of asynchronously growing Chinese hamster ovary (CHO) cells grown *in vitro* and to the cytokinetic analysis of mouse bone marrow cells from mice treated *in vivo* with 1-β-D-arabinofuranosylcytosine (araC).

MATERIALS AND METHODS

***In Vitro* Studies.** In one series of experiments, CHO cells growing exponentially in minimal essential alpha medium with

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Abbreviations: araC, 1-β-D-arabinofuranosylcytosine; CHO, Chinese hamster ovary; P_i/NaCl, phosphate-buffered saline; PrdI, propidium iodide.

10% fetal calf serum at 3×10^5 cells per ml were treated for 30 min with 10 μM BrdUrd. The cells were then refed with conditioned medium from a second untreated spinner flask. Twenty-milliliter aliquots were removed at 2-hr intervals, counted, washed with phosphate-buffered saline (P_i/NaCl, pH 7.2), and fixed for at least 30 min in cold 70% ethanol.

In a second series of experiments, exponentially growing CHO cells were treated with [^{3}H]BrdUrd for 30 min (specific activity, 35 Ci/mmol; 1 Ci = 3.7×10^{10} Bq; final concentration, 1 μCi/ml). The cells were then harvested, washed in P_i/NaCl, and fixed for at least 30 min in cold 70% ethanol.

***In Vivo* Studies.** Control and araC-treated female C3H mice (Washington University, St. Louis, MO) were injected intraperitoneally with 50 mg of BrdUrd per kg 1 hr prior to sacrifice. araC-treated mice received BrdUrd 3, 6, and 9 hr after a single intraperitoneal injection of 500 mg of araC (Upjohn) per kg. The femurs of each mouse were removed and the hematopoietic cells were harvested by flushing with 1 ml of alpha medium. The recovered cells were then resuspended by pipetting, filtered through 37-μm mesh, and fixed with 70% ethanol.

Cytochemistry. Cells were removed from ethanol (in which they can be stored for several days at 4°C), resuspended in 2 ml of 1.5 M HCl at 20°C for 20 min, washed twice with 5 ml of P_i/NaCl, and resuspended for 1 hr in P_i/NaCl solution containing 0.5% Tween 20 (Sigma), 0.5% bovine serum albumin, and a 1:200 dilution of monoclonal anti-BrdUrd (8). The cells were then washed twice with P_i/NaCl and resuspended for 30 min in a solution containing P_i/NaCl, 0.5% Tween 20, a 1:100 dilution of a stock solution of fluorescein-labeled goat anti-mouse gamma globulin (Cappel Laboratories, Cochranville, PA), and 1% neutral goat serum (to prevent nonspecific adsorption of the fluorescein-labeled anti-mouse gamma globulin). The cells were then washed twice with 5 ml of P_i/NaCl and resuspended in 1 ml of P_i/NaCl containing 5 μg of PrdI (Polysciences, Warrington, PA) per ml. After 1 hr, the cells were ready for flow cytometric analysis.

Flow Cytometry and Sorting. Cell analysis was performed with the Lawrence Livermore National Laboratory flow cytometer (9). Cell sorting was accomplished by using a fluorescence-activated cell sorter (Becton Dickinson, Sunnyvale, CA). During flow cytometry, cells were excited at 488 nm. Red fluorescence from PrdI was collected through a 600-nm-long wavelength pass filter and recorded as a measure of total DNA content and green fluorescence from fluorescein was collected through a 514-nm bandpass filter and recorded as a measure of the amount of incorporated BrdUrd. The resulting data were accumulated to form a bivariate 64 × 64 channel distribution showing the distribution of DNA (red fluorescence) and BrdUrd (green fluorescence) among the cells of the population.

Cells were sorted from several regions of the bivariate DNA/BrdUrd distribution measured for CHO cells treated for 30 min with [^{3}H]BrdUrd. Sorting regions are indicated in Fig. 2 *Upper*. Ten thousand cells were sorted from each region directly into liquid scintillation vials. The radioactivity per cell was determined as described (10).

RESULTS

***In Vitro* Studies.** Fig. 1 shows bivariate DNA/BrdUrd distributions measured for CHO cells before BrdUrd treatment (*Left*) and immediately after a 30-min BrdUrd pulse (*Right*). The G_1-, S-, and G_2M-phase cells in the unlabeled population are all seen at low green fluorescence as are G_1- and G_2M-phase cells in the labeled population. However, the S-phase cells in the labeled population have substantial green fluorescence. Gratzner and Leif (11) have reported that anti-BrdUrd antibodies bind stoichiometrically to BrdUrd in DNA so, after correction for low-level nonspecific staining, the intensity of green fluorescence is proportional to the amount of incorporated BrdUrd. This procedure is highly sensitive so that low levels of BrdUrd incorporation can be easily quantified. In fact, we have found that BrdUrd incorporated during a 3-min pulse can be easily quantified by this procedure (data not shown). This antibody-based procedure is substantially more sensitive than earlier flow cytometric procedures proposed for BrdUrd measurements, in which BrdUrd incorporation was assessed by the

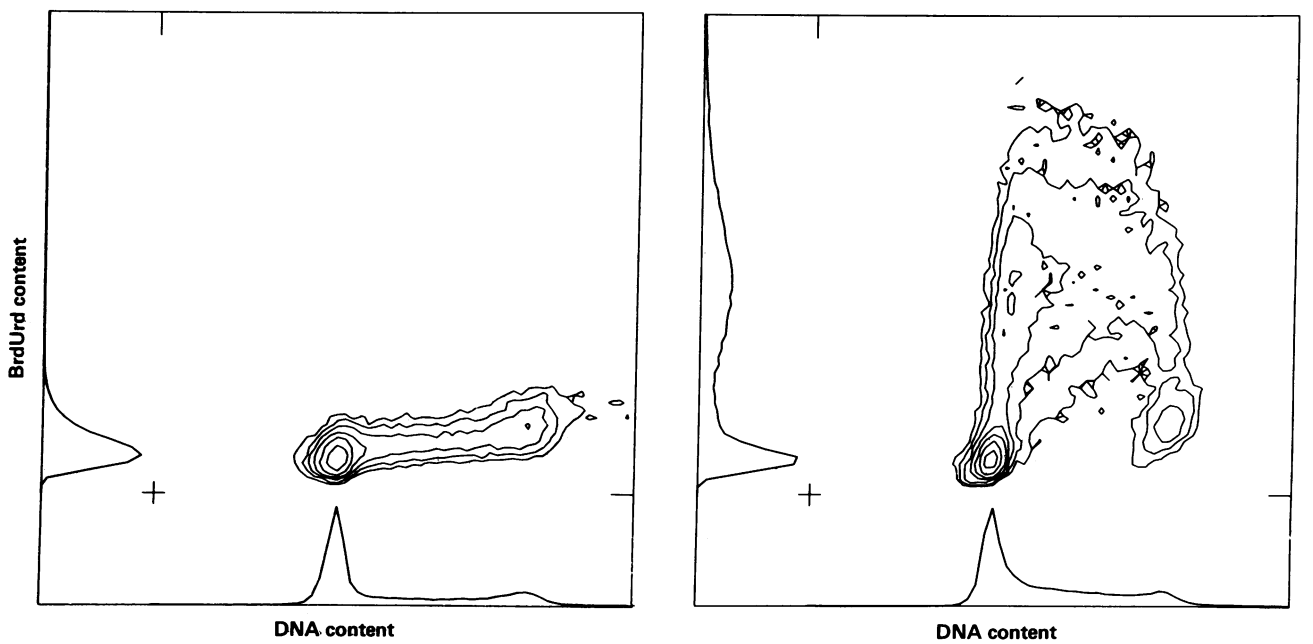

FIG. 1. Contour plots of bivariate DNA/BrdUrd distributions measured for exponentially growing CHO cells prior to treatment with BrdUrd (*Left*) and immediately after a 30-min exposure to 10 μM BrdUrd (*Right*). The origin of each contour plot is indicated with a cross. Also shown on the abscissas and ordinates are univariate frequency distributions for total DNA content (red fluorescence) and amount of incorporated BrdUrd (green fluorescence), respectively.

extent of quenching of Hoechst 33258 fluorescence (12, 13). The earlier procedure could be applied only to quantification of BrdUrd incorporated over a period of several hours.

The bivariate distributions provide detailed information about the kinetics of the DNA synthesis process. For example, the DNA/BrdUrd distribution in Fig. 1 measured for CHO cells immediately after pulse labeling with [^{3}H]BrdUrd clearly shows the variation in BrdUrd incorporation rates. Substantial variation in BrdUrd-linked green fluorescence is seen, even for cells with the same DNA content. These data also show the variation in the rate of BrdUrd incorporation across S phase. They show the rate of BrdUrd incorporation to be highest in mid-S phase and lowest in early and late S phase and are in good agreement with previous measurements of the variation of the rate of incorporation of [^{3}H]dThd across S phase (10).

The relation between the amount of incorporated BrdUrd and the intensity of green fluorescence is illustrated by data in Fig. 2 *Lower*, which shows the correlation between BrdUrd-linked green fluorescence and the amount of [^{3}H]BrdUrd per cell as determined by liquid scintillation spectrometry for cells sorted from the regions marked in Fig. 2 *Upper*. The correlation between BrdUrd-linked green fluorescence and BrdUrd incorporation is good ($r = 0.97$), confirming that in CHO cells the green fluorescence is an accurate measure of BrdUrd incorporation.

Cell cycle traverse rates can be estimated from DNA/BrdUrd distributions measured periodically after BrdUrd pulse labeling, such as those shown in Fig. 3 for CHO cells. These data show that the BrdUrd-labeled cohort, initially in S phase, begins to move out of S phase and through G_2M immediately after labeling. By 8 hr, the labeled cells are predominantly in G_2M phase and G_1 phase, and by 10 hr they have begun to reenter S phase. The magnitude of the green fluorescence halves as the labeled cohort passes through division because the average amount of BrdUrd per cell halves. The BrdUrd content per cell in mid-S phase is high immediately after labeling and decreases and increases as the BrdUrd-labeled cohort moves out of and back into S phase. The BrdUrd content per cell in G_1 phase is low immediately after labeling and increases and decreases as the labeled cohort moves into and out of G_1 phase. In practice, we estimate cell cycle phase durations quantitatively from changes in the average values of BrdUrd per cell in mid-S phase and BrdUrd per cell in G_1 phase extracted from the bivariate distributions. These curves are quantitatively the same as the radioactivity per cell in mid-S-phase and G_1-phase curves measured for cells sorted from samples labeled with a radioactive DNA precursor and can be analyzed by using the same computer analysis procedure (14). The G_1-, S-, and G_2M-phase durations estimated for the CHO cells by this procedure are 7.2, 7.4, and 1.1 hr, respectively.

***In Vivo* Studies.** Bivariate DNA/BrdUrd analyses can also be carried out *in vivo* for normal and araC-treated populations. Fig. 4 shows bivariate DNA/BrdUrd distributions measured for mouse bone marrow cells obtained from mice pulse labeled with BrdUrd 3, 6, and 9 hr after treatment with 500 mg of araC per kg. The control bivariate distribution was obtained from mice that received only BrdUrd 30 min prior to sacrifice. All distributions show two distinct peaks with G_1 DNA content—one at low green fluorescence and one at higher green fluorescence. Cells sorted from the low green fluorescence peak were determined by microscopic analysis to be lymphocytes and blast cells, whereas cells sorted from the high green fluorescence peak were determined to be mostly well-differentiated granulocytes and macrophages. In the control population, cells sorted from mid-S phase were determined to be predominantly blast cells and thus to be associated with the low green fluorescence

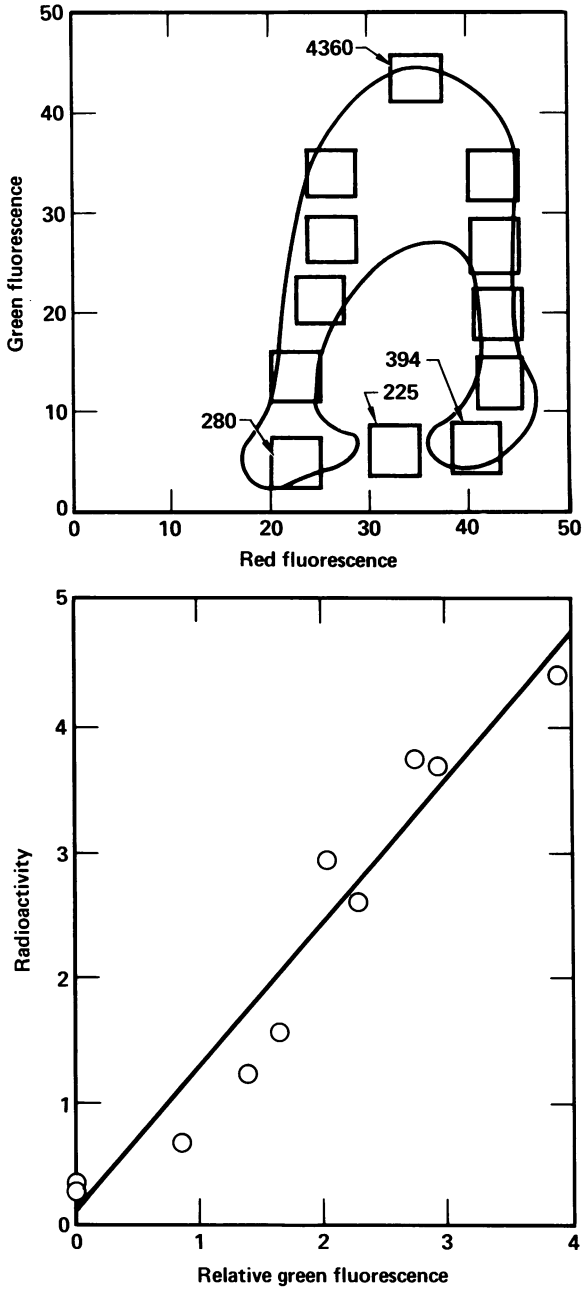

FIG. 2. (*Upper*) A bivariate DNA/BrdUrd distribution measured for exponentially growing CHO cells immediately after a 30-min treatment with [^{3}H]BrdUrd. For simplicity, only a single contour level is shown to outline the bivariate distribution. Also shown are regions from which cells were sorted for determination of the radioactivity per cell. (*Lower*) A plot showing the correlation between BrdUrd-linked green fluorescence and radioactivity per cell for the various regions shown in *Upper*. Background levels of green fluorescence were subtracted before linear regression analysis. The solid line is a linear regression to the data.

peak with G_1-phase DNA content. The DNA/BrdUrd distribution measured for cells treated with BrdUrd 3 hr after araC treatment shows that the majority of cells with an S-phase DNA content are characterized by low green fluorescence values, indicating negligible levels of DNA synthesis (i.e., BrdUrd incorporation). However, the DNA/BrdUrd distribution measured for cells treated with BrdUrd 6 hr after araC treatment shows that most of the cells with S-phase DNA content that did not incorporate BrdUrd are gone (perhaps lost to phagocytosis

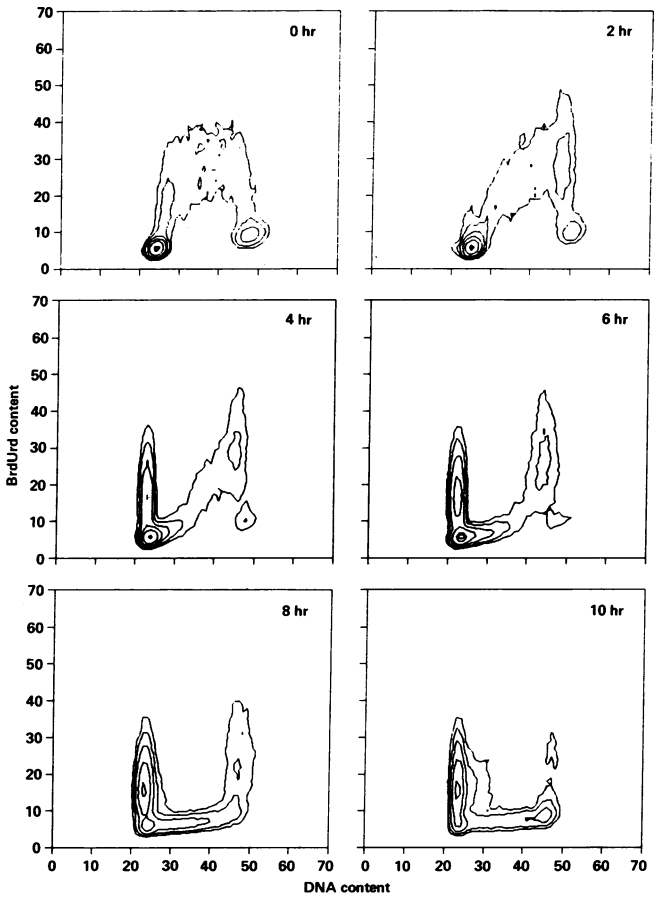

FIG. 3. Bivariate DNA/BrdUrd distributions measured for exponentially growing CHO cells at 2-hr intervals after a 30-min treatment with 10 μM BrdUrd. Data shown as described in the legend to Fig. 1.

or migration). The appearance in early S phase of a population incorporating substantial amounts of BrdUrd is also evident in this distribution. The DNA/BrdUrd distribution measured for cells treated with BrdUrd 9 hr after araC treatment shows a substantial population of cells in early S phase that have incorporated a greater than normal amount of BrdUrd, suggesting an elevated rate of DNA synthesis.

DISCUSSION

We have demonstrated in this paper a technique for the simultaneous flow cytometric analysis of cellular DNA content and amount of incorporated BrdUrd. The critical aspect of the procedure is the use of 1.5 M HCl to *partially* denature the cellular DNA so that the monoclonal anti-BrdUrd can bind to the incorporated BrdUrd (the antibody does not bind to BrdUrd in double-stranded DNA; data not shown) while leaving sufficient double-stranded DNA for PrdI binding. The HCl treatment also serves to hydrolyze double-stranded RNA, so that treatment with RNase is not required to get highly DNA-specific PrdI binding. PrdI fluorescence distributions measured for CHO cells treated with HCl, as described in this report, or treated with RNase, as described by Gray and Coffino (15), were similar in shape, illustrating that the HCl treatment did not substantially alter the PrdI fluorescence distribution. In addition, the fluorescence intensity was not substantially different for HCl vs. RNase treatment. However, inferences about the extent of DNA denaturation cannot be made from these data because the hydrolysis treatment is expected to remove histone proteins, thereby increasing the number of available PrdI binding sites (16), while denaturing DNA, thereby decreasing the number of PrdI binding sites (17).

The good correlation between BrdUrd-linked green fluorescence and amount of radioactivity per cell for cells sorted from a [^{3}H]BrdUrd-labeled cell population (Fig. 2 *Lower*) illustrates that, for CHO cells, the BrdUrd-linked green fluorescence is proportional to the amount of incorporated BrdUrd. In spite of this, some of the distributions in Figs. 1–3 show elevated green fluorescence intensities for cells with apparent G_1 DNA content. In the DNA/BrdUrd distribution in Fig. 1, the elevated green fluorescence for some cells with G_1 DNA content is a reflection of the sensitivity of the BrdUrd labeling procedure and is not due to nonspecific antibody binding. The elevated green fluorescence results from antibody binding to BrdUrd incorporated during only a few minutes of DNA synthesis as cells moved into S phase midway during the labeling period. The total DNA content change during this short synthetic period is so small that no change can be detected in total DNA content. This can be understood by realizing that in CHO cells with a 7-hr S phase (and assuming, for simplicity, a constant rate of DNA synthesis), a 15-min period of DNA synthesis would cause only a 4% increase in total DNA content (a 1-channel shift in the distribution in Fig. 1). Therefore, it is not surprising that some cells with an apparent G_1 DNA content actually are early S-phase cells and have incorporated measurable amounts of BrdUrd. The elevated green fluorescence in cells with G_1-phase DNA content in the 4-hr distribution in Fig. 3 is also a quantitative measure of BrdUrd incorporation. Here the distribution shows the BrdUrd-labeled cohort of cells that moved out of S phase and into G_1 in the 4-hr period after the BrdUrd labeling period. Thus, all of the *in vitro* data supports our conclusion that we have developed a quantitative procedure for the flow cytometric measurement of cellular DNA content and amount of incorporated BrdUrd.

Interpretation of the *in vivo* study of mouse bone marrow is more complicated. In this study, a population of G_1 cells was identified that seemed to bind antibody nonspecifically. These cells were sorted and determined morphologically to be highly differentiated cells. Such cells are known to possess F_c receptors and thus bind immunoglobulins nonspecifically (18). Such nonspecific binding should be taken into account during interpretation of bivariate DNA/BrdUrd distributions measured for bone marrow populations.

The DNA/BrdUrd analysis procedure described in this paper should facilitate a broad range of biological and biomedical investigations. The *in vitro* CHO studies illustrate the utility of the procedure in studies of DNA synthesis and in tracing the movement of a BrdUrd-labeled cohort of cells around the cell cycle, as required for cytokinetic analysis of asynchronous populations (Fig. 3). The *in vivo* studies of bone marrow cells before and after treatment with araC demonstrate the applicability of the procedure for *in vivo* analyses and suggest the power of the technique in the study of drug-treated populations (Fig. 4). The procedure appears especially useful for discrimination between therapeutically inactivated cells with S-phase DNA content and cells in S phase that are actively synthesizing DNA.

The BrdUrd procedure should also be of use to oncologists interested in the detection of low-frequency hyperdiploid tumor populations (19). After BrdUrd labeling, hyperdiploid G_1 populations, normally obscured by diploid S-phase cells, should be visible in the DNA/BrdUrd distribution as having elevated DNA content and low BrdUrd content; the normal S-phase cells should fall in a different portion of the DNA/BrdUrd distribution, having elevated DNA contents and elevated BrdUrd contents.

The DNA/BrdUrd procedure has several advantages over

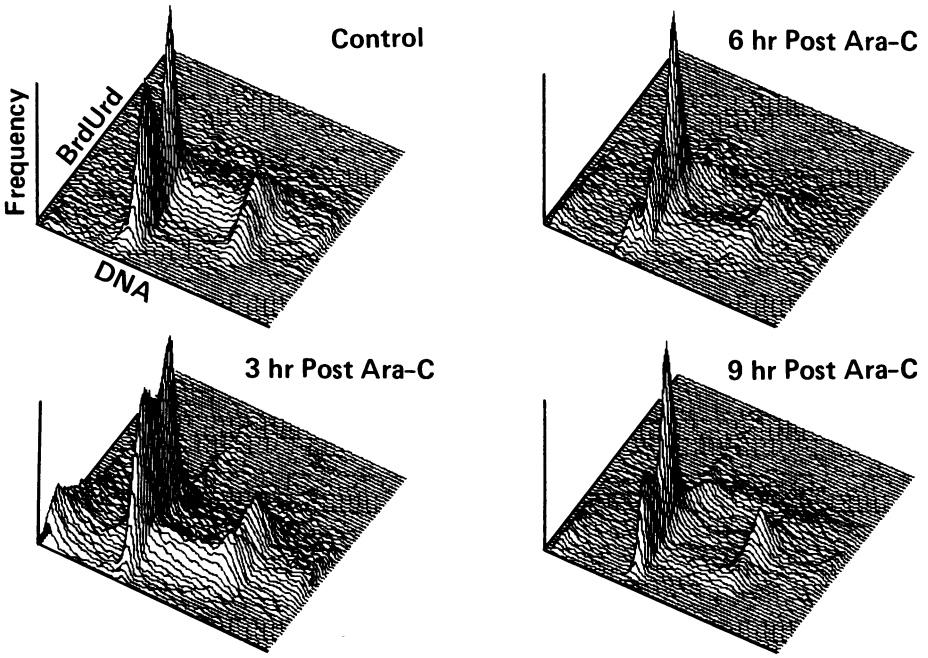

FIG. 4. Bivariate DNA/BrdUrd distributions measured for bone marrow cells obtained from control C3H mice and from C3H mice labeled with BrdUrd 3, 6, or 9 hr after araC treatment. Data are shown as isometric plots with DNA content on the *x* axis, BrdUrd on the *y* axis, and frequency$^{1/2}$ on the *z* axis. The square root of the frequency is displayed to show the low frequency populations in S phase more clearly.

previously reported procedures for study of cell proliferation or DNA synthesis. (*i*) Radioactive DNA precursors are not required so that problems associated with radiotoxicity are eliminated. Of course, this may be replaced by some BrdUrd toxicity. However, the magnitude of this is unknown and may be decreased by simultaneous treatment with deoxycytidine. (*ii*) The analysis time is short (as low as 5 min per sample) and only a few million cells are required for analysis. (*iii*) The difficulty of discriminating between labeled and unlabeled cells inherent in autoradiography is substantially decreased because the distribution of background green fluorescence for unlabeled G_1-, S-, and G_2M-phase cells can be accurately measured and taken into account during analysis of bivariate DNA/BrdUrd distributions. (*iv*) Discrimination can be made between labeled and unlabeled cells with S-phase DNA content. This feature is especially useful during the study of perturbed populations. (*v*) These measurements can be performed by using relatively simple, commonly available flow cytometers.

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1. Hart, J. S., George, S. L., Frei, E., III, Bodney, G. P., Nickerson, R. C. & Freireich, E. J. (1977) *Cancer* **39**, 1603–1617.
2. Barranco, S. C., May, J. T., Boerwinkle, W., Nichols, S., Hokanson, K. M., Schumann, J., Göhde, W., Bryant, J. & Guseman, L. F. (1982) *Cancer Res.* **42**, 2894–2898.
3. Quastler, H. & Sherman F. (1959) *Exp. Cell Res.* **17**, 420–429.
4. Pallavicini, M. G., Gray, J. W. & Folstad, L. J. (1982) *Cancer Res.* **42**, 3125–3131.
5. Painter, R. B. & Young, B. R. (1976) *Biochim. Biophys. Acta* **418**, 146–153.
6. Simpson-Herren, L., Danford, A. H., Holmquist, J. P., Springer, T. A. & Lloyd, H. H. (1976) *Cancer Res.* **36**, 4705–4709.
7. Gray, J. W., Dean, P. N. & Mendelsohn, M. L. (1979) in *Flow Cytometry and Sorting*, eds. Melamed, M., Mullaney, P. L. & Mendelsohn, M. (Wiley, New York), pp. 383–407.
8. Gratzner, H. (1982) *Science* **218**, 474–475.
9. Peters, D., Dean, P. & Pinkel, D. (1981) *Lawrence Livermore National Laboratory Report UCID-19260* (Lawrence Livermore National Laboratory, Livermore, CA).
10. Gray, J. W., Pallavicini, M. G., George, Y. S., Groppi, V., Look, M. & Dean, P. N. (1981) *J. Cell. Physiol.* **108**, 135–144.
11. Gratzner, H. G. & Leif, R. C. (1981) *Cytometry* **1**, 385–389.
12. Latt, S. A., George, Y. & Gray, J. W. (1977) *J. Histochem. Cytochem.* **25**, 927–934.
13. Bohmer, R. & Ellwort, J. (1981) *Cytometry* **2**, 31–34.
14. Gray, J. W., Bogart, E., Gavel, D. T., George, Y. S. & Moore, D. H. (1982) *Cell Tissue Kinet.*, in press.
15. Gray, J. W. & Coffino, P. (1979) *Methods Enzymol.* **58**, 233–248.
16. Traganos, F., Darzyniewicz, Z., Sharpless, T. & Melamed, M. R. (1976) *J. Histochem. Cytochem.* **24**, 40–48.
17. Le-Pecq, J. B. & Paoletti, C. (1967) *J. Mol. Biol.* **27**, 87–106.
18. Hood, L. A., Weissman, J. L. & Wood, W. B. (1978) in *Immunology*, eds. Hood, L. A., Weissman, I. & Wood, W. B. (Benjamin/Cummings, Menlo Park, CA), p. 467.
19. Barlogie, B., Buchner, T., Hart, J. S., Ahearn, M. J. & Freireich, E. J. (1975) in *Pulse-Cytophotometry, First International Symposium on Pulse Cytophotometry*, eds. Haanen, C. A. M., Hillen, H. F. P. & Wessels, J. M. C. (European Press, Ghent, Belgium), pp. 299–305.