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INTRODUCTION

Clonogenic assays (1) have been used extensively in the assessment of chemosensitivity of both established tumor cell lines (2) and freshly biopsied tumor tissue (3, 4). Low plating efficiencies, clumping artifacts, and assay duration are technical problems that limit the wide spread applicability of clonogenic assays on human tumor material (5-7). In an attempt to overcome the limitations of the clonogenic assay, a number of short-term assays have been developed, including dye exclusion techniques (8), tritiated thymidine uptake (9), radiolabeled glucose utilization (10), and automated image analysis of crystal violet-stained cells (11). However, there are conflicting views regarding the comparability of these assays to the clonogenic assay (12-15).

Recently, changes have been proposed in the National Cancer Institute’s anticancer drug screening program. The first involves the use of human tumor cell lines in a disease-oriented approach (16). Thus, a large panel of human lung cancer lines representing the major histological types (including non-small cell and small cell lung cancer) will be screened. The second involves the use of a colorimetric assay based on the ability of live but not dead tumor cells to reduce a tetrazolium-based compound (MTT) to a blue formazan product. Chemosensitivity assays using tetrazolium dyes have been widely reported (17, 18) and are in current use in Japan. With modification this assay is attractive in that it can be semiautomated (19), because the assay can be performed in 96-well plates, with MTT formazan production analyzed using a scanning multiwell spectrophotometer. Thus large numbers of samples can be analyzed simply and rapidly. Recently, further modification of this assay has been reported (20). However, comparison of the MTT assay with other assays is essential prior to its use in large scale screening studies. Therefore, direct comparisons were made between the clonogenic, dye exclusion, and MTT assays utilizing both non-small cell and small cell lung cancer cell lines. In addition Chinese hamster V79 cells and 2 Chinese hamster ovary cell lines, the wild type AuxB1 and its pleiotropic drug mutant CHRC5 (21), were also tested. These cell lines were assessed for sensitivity to a range of cytotoxic drugs and to radiation, with the details of the radiation experiments described in the following article (22). First we discovered the need to significantly modify the previously described MTT assay (19) to allow for better solubilization of the formazan product. Using this modification we were then able to show that the MTT assay gives results comparable to those of the clonogenic and dye exclusion assays for lung cancer and Chinese hamster cells. Based on these studies it appears reasonable to use the MTT assay for new drug screening.

MATERIALS AND METHODS

Cell Lines

Hamster Cell Lines. Chinese hamster lung fibroblast V79 cells were grown as a monolayer in Ham’s F-12 medium (Gibco) supplemented with 10% (v/v) fetal calf serum, penicillin, and streptomycin. Exponentially growing cultures were maintained in a humidified atmosphere of 5% CO2-95% air at 37°C, and under these conditions the plating efficiency was 70-90% and the doubling time was 9-10 h. Two Chinese hamster ovary cell lines kindly provided by Dr. V. Ling were used: CHO-AuxB1 and its pleiotropically drug-resistant mutant CHRC5 (21). These two lines were grown as monolayers under the same conditions as for V79 cells, and plating efficiencies in both cell lines ranged from 60 to 75%. Under these conditions doubling times were 13-15 h for CHO-AuxB1 and 22 h for CHRC5.

Human Lung Cancer Cell Lines. Two human lung cancer cell lines were used: NCI-H249, a small cell lung cancer, and NCI-H460, a large cell lung carcinoma cell line. Exponentially growing cultures of both cell lines were grown in RPMI 1640 (Gibco) supplemented with 10% (v/v) fetal calf serum, penicillin, and streptomycin and were maintained in a humidified atmosphere of 7% CO2-93% air at 37°C. NCI-H460 grew as a monolayer with a doubling time of 20-25 h and plating efficiency of 50-70%, whereas NCI-H249 grew as floating aggregates (23), with a doubling time of 85 h and cloning efficiency in soft agar of 2%.

MTT Assay

Single cell suspensions were obtained by mechanical disaggregation of the floating cell line and by trypsinization of monolayer cultures, with cell counts performed using an Elzone particle counter (Particle Data, Inc., Elmhurst, IL). The assay is dependent on the cellular reduction of MTT (Sigma Chemical Co., St. Louis, MO) by the...
mitochondrial dehydrogenase of viable cells to a blue formazan product which can be measured spectrophotometrically. Following appropriate incubation of cells, with or without drug, 0.1 mg (50 µl of 2 mg/ml) MTT was added to each well and incubated at 37°C for a further 4 h before processing as described below. To determine the relationship of cell number to MTT formazan crystal formation increasing cell numbers from 100 to 100,000/well were plated, using V79, NCI-H460, and NCI-H249 cells. MTT was immediately added and plates were incubated for 4 h. Plates were then processed as described and absorbance was determined. For cell growth studies, serially increasing cell numbers were plated in different columns across 96-well microtiter plates. Actual cell number was measured daily by removing cells from individual wells at each cell concentration. Cell counts were performed using a particle counter, with all measurements carried out in triplicate. Identical plates were processed daily using MTT, measuring absorbance with a scanning multwell spectrophotometer (enzyme-linked immunosorbent assay reader; Biotek Instruments, Inc., Burlington, VT).

Growth rates varied between the cell lines used; therefore, the number of cells plated was adjusted so that untreated cells were in exponential growth phase at the time of harvest. An incubation time of 4 days was selected for a number of reasons: (a) this allowed sufficient time for drug-induced cell death to occur; (b) this enabled us to directly compare results of this assay with data assimilated by dye exclusion assay, which has been widely used in this laboratory; and (c) by keeping the incubation time short, this obviated the need to refeed cells, maximizing the benefit of the automation of the assay. In view of the rapid doubling time of V79 cells, a 72-h incubation was considered optimal, because longer incubation times would have necessitated the seeding of less than 100 cells to ensure logarithmic growth at the end of the experiment.

Assays were performed using both 1-h and continuous exposure to drugs. For continuous exposure studies, equal numbers of cells were inoculated into each well in 0.18 ml of culture medium, to which 0.02 ml of 10× concentrated drug or phosphate-buffered saline was added. For 1-h exposure, drugs and cells were mixed in 15 x 75-mm test tubes, and following incubation, cells were centrifuged at 250 x g for 5 min, washed twice with phosphate-buffered saline, and then plated directly into microtiter plates. Following incubation the media from plates containing adherent cells was aspirated completely, whereas for the floating cell line, NCI-H249, plates were centrifuged at 450 x g for 5 min in a plate holder. The majority of the media was then aspirated, taking care not to disturb the formazan crystals, leaving approximately 30 µl of media in each well. All results represent the average of a minimum of 8 wells. Surviving fraction or fractional absorbance was calculated by the formula: mean of test sample + mean of untreated sample. Additional controls consisted of media alone with no cells, with or without the various drugs.

Using the procedure described by Mosmann (19) with acid isopropyl alcohol as solvent, we were unable to achieve solubilization of the formazan crystals, and minimal absorbance was detected at 570 nm. A modification of this technique has recently been described (20). However, because it is proposed to use this assay for human tumor cell line screening, this modification was far from ideal. Many of these cells require serum for optimal growth and a number grow as floating cell lines, or 4 days for all other cell lines. Following incubation, 50,000 aldehyde-fixed duck RBC were added to each tube and the cells were centrifuged at 37°C for 3 days in view of the rapid doubling time in experiments using V79 cells, or 4 days for all other cell lines. Following incubation, 50,000 aldehyde-fixed duck RBC were added to each tube and the cells were centrifuged at a volume of 0.2 ml by centrifugation at 250 x g for 5 min. The tubes were then vortexed, 0.2 ml of a 1% fast green-0.5% nigrosin solution was added, and tubes were again vortexed. After 10 min of incubation, cell suspensions were vortexed and cytocentrifuged at 1,200 x g for 7 min. The cell discs were then fixed in methanol and counterstained with hematoxylin and eosin. Cells were counted at ×400 using a light microscope. The viable (hematoxylin-eosin-stained) tumor cell/duck RBC ratio was determined for each treatment group and compared to control values.

Clonogenic Assay

Clonogenic assays were performed in standard fashion, using previously described techniques (3, 24, 25). Drug treatment was administered either by 1-h exposure, following which cells were rinsed free of drug using phosphate-buffered saline prior to plating, or by continuous exposure, with triplicate cultures plated at each drug concentration. Adherent cell lines were plated directly onto Petri dishes and incubated with or without drug for variable times, dependent on the growth rate of the cells. One hundred V79 cells were incubated for control plates, with higher cell numbers used for treated samples to give at least 50 colonies and plates incubated for 6 days. Incubation times were 7 days for CHO cell lines and 10 days for NCI-H460 cells. For these cell lines control plates were inoculated with 250 cells/dish, with higher cell numbers used for drug-treated samples. Colonies were fixed in methanol-acetic acid (3:1), stained with crystal violet, and counted using a microscope at ×40. For all clonogenic assays colonies of greater than 50 cells were counted, with cell inocula of 5 x 10⁴ cells or less plated on 60-mm Petri dishes and larger cell inocula plated on 100-mm Petri dishes. NCI-H249, the floating cell line, was suspended in 0.6% agar and plated onto a feeder layer of 0.6% agar, with the medium used in both agar layers consisting of RPMI 1640 plus 10% (v/v) fetal calf serum. Experiments were performed using 3 ml for each agar layer in 60-mm dishes and 8 ml for each agar layer in 100-mm dishes, with all plates incubated for 18 days.

Drugs

All drugs were obtained from commercial sources apart from melphanal which was obtained from the Drug Synthesis and Chemistry Branch of the National Cancer Institute. Drugs were prepared every 2 months and stored at 100× concentrations at −70°C, apart from melphanal which was prepared freshly for each experiment.

Study Design

All three assays were set up simultaneously for each cell line, using the same drug solutions. For 1-h drug exposure, cells were incubated with drug in a test tube, then washed free of drug, and plated directly in both Petri dishes and 96-well plates. Survival or fractional absorbance was expressed as a percentage of control values. For clonogenic studies this means that the number of colonies surviving a given treatment were compared to untreated control plates. For the MTT assay, absorbance levels from drug-treated cells were corrected against untreated control absorbance values. For the dye exclusion assay, the ratio of viable tumor cells to duck RBC in treated specimens was compared with the same ratio in control tubes. The ID₅₀ was defined as 50% reduction in colony number in the clonogenic assay, 50% reduction of absorbance in the MTT assay, or 50% reduction in the tumor/duck RBC ratio using the dye exclusion assay.

Standard errors were calculated according to the method of Bevington (26) which takes into account the error for each experimental point.
and the error associated with control values. Error bars are shown on the figure when larger than the diameter of the symbol.

RESULTS

Modification of the MTT Assay to Give Better Solubilization of the MTT Formazan Product. The solubility of the MTT-reduced formazan product was poor in acid isopropyl alcohol as used in the method of Mosmann (19), while better absorption characteristics were observed with mineral oil and DMSO as solvents (Fig. 1). From these experiments we chose 570 nm for reading the mineral oil solubilized product. DMSO solubilization gave an absorption peak ($\lambda_{\text{max}}$) at 503 nm. However, $\lambda_{\text{max}}$ has been shown to vary considerably for MTT formazan, dependent on the solvent used, and other factors such as the concentration of metal ions in the solution (27). With floating cultures, when all the media was not removed, there was a shift in the $\lambda_{\text{max}}$, with maximal absorbance detected reproducibly at 540 nm. Therefore, this wavelength was used for floating cultures, with DMSO as the solvent.

MTT Assay: Effect of Cell Number on MTT Formazan Production. The amount of formazan product generated and then measured after solubilization in mineral oil or DMSO at 570 or 540 nm is proportional to cell number, although absolute absorbance for a given cell number varies between cell lines (Fig. 2). These data were generated by plating cells at the inoculum noted on the x-axis. Cells were then immediately incubated with MTT for 4 h, and the plates were then processed. There was good reproducibility between replicate wells with standard errors $\pm 10\%$. The growth rates of cell lines in microtiter wells were then determined using the MTT assay (Fig. 3). Individual wells were analyzed from each cell line, and cells were counted revealing doubling times of 10 h in V79 cells, 26 h in NCI-H460 cells, and 85 h in NCI-H249 cells. These results are comparable to doubling times obtained by standard methods in these cell lines (data not shown). For each cell line optimal seeding concentrations were derived, to give where possible maximal absorbance while ensuring that cells remained in exponential growth. The cell number chosen for V79 cells was 100 cells/well for a 3-day assay and for NCI-H460 2,500 and 10,000 cells/well using a 4-day assay. Metabolism of MTT was poor in NCI-H249 cells, with low absorbance values. However, because culture conditions such as cell density can significantly affect the results of clonogenic assays, we chose to keep seeding cell numbers similar between assays. Therefore, despite low absorbance values, the 10,000-NCI-H249 cells/well sample was chosen as the optimal inoculum.

Assessment of Drug Resistance in CHO Pleiotropic Mutant Cells. Chemosensitivity was assessed by continuous exposure...
Comparison of Assays Using Continuous Drug Exposure. The three cell lines (V79, NCI-H460, and NCI-H249) were used to compare all three assays following continuous exposure to five drugs, doxorubicin (Adriamycin), vinblastine, cisplatin, melphalan, and etoposide, in 2 CHO lines, the wild-type AuxB1, and its pleiotropic mutant CH\textsuperscript{PC5}. The latter was made resistant to colchicine and has been shown to exhibit a typical cross-resistance pattern to many drugs (21). Clonogenic and MTT assays were carried out simultaneously in these cells and the results are illustrated in Fig. 4, with ID\textsubscript{50}s shown in Table 1. As can be seen in Fig. 4, a high degree of resistance to Adriamycin and vinblastine is exhibited by the CH\textsuperscript{PC5} cell line, with the relative degree of resistance between cell lines similar using both assays. There is little cross-resistance to cisplatin, and this is exemplified by both assays. Some cross-resistance to melphalan is exhibited with quantitatively similar results obtained with both assays. The clonogenic assay was more sensitive in all experiments, probably relating to the longer duration of drug exposure with this assay. The relative degree of resistance observed for each drug using the clonogenic technique was maintained using the MTT assay.

Comparison of Assays following 1-h Drug Exposure. Comparisons were made of the MTT assay and clonogenic assay in the single agents Adriamycin, vinblastine, etoposide, and cisplatin. Each drug was tested at a minimum of three concentrations, and the ID\textsubscript{50}s were determined as shown in Table 2. For adherent cell lines, excellent correlation was observed for ID\textsubscript{50}s following continuous exposure to cisplatin, Adriamycin, and etoposide, although nonclonogenic assays appeared more sensitive to vinblastine.

With cisplatin good correlation was observed for all cell lines in the three assays. Nonclonogenic assays gave higher ID\textsubscript{50}s for Adriamycin and etoposide, using the floating cell line NCI-H249, but it should be noted that drug exposure time was 18 days for the clonogenic assay and 4 days for nonclonogenic assays. Dose-response curves were generated for all 4 drugs using the 3 assays with representative examples illustrated in Fig. 5, which shows that reasonable correlation is observed between the MTT and clonogenic assays. The clonogenic assay appeared to be more sensitive, although allowance should be made for increased drug exposure time in the clonogenic assays, particularly those using NCI-H249 cells.

Comparison of Assays following 1-h Drug Exposure. Comparisons were made of the MTT assay and clonogenic assay in the

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**Table 1** ID\textsubscript{50} of 2 CHO cell lines, wild type AuxB1 and its pleiotropic mutant CH\textsuperscript{PC5}, following continuous exposure to various drugs using the clonogenic assay and the MTT assay

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Adriamycin (nM) CA</th>
<th>MTT</th>
<th>Vinblastine (nM) CA</th>
<th>MTT</th>
<th>Etoposide (nM) CA</th>
<th>MTT</th>
<th>Cisplatin (nM) CA</th>
<th>MTT</th>
<th>Melphalan (nM) CA</th>
<th>MTT</th>
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</thead>
<tbody>
<tr>
<td>AuxB1 (WT)</td>
<td>35</td>
<td>45</td>
<td>12.5</td>
<td>9.5</td>
<td>400</td>
<td>450</td>
<td>1,000</td>
<td>1,100</td>
<td>50</td>
<td>28</td>
</tr>
<tr>
<td>CH\textsuperscript{PC5}</td>
<td>5,000</td>
<td>10,000</td>
<td>650</td>
<td>435</td>
<td>5,000</td>
<td>10,000</td>
<td>1,000</td>
<td>2,800</td>
<td>400</td>
<td>300</td>
</tr>
<tr>
<td>Resistance factor</td>
<td>140\times</td>
<td>220\times</td>
<td>52\times</td>
<td>45\times</td>
<td>37.5</td>
<td>22\times</td>
<td>1\times</td>
<td>2.5\times</td>
<td>8\times</td>
<td>11\times</td>
</tr>
</tbody>
</table>

* CA, clonogenic assay.

**Table 2** ID\textsubscript{50} following continuous exposure to various drugs using the clonogenic assay, the dye exclusion assay, and the MTT assay

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Adriamycin (nM) CA</th>
<th>DEA</th>
<th>MTT</th>
<th>Vinblastine (nM) CA</th>
<th>DEA</th>
<th>MTT</th>
<th>Etoposide (nM) CA</th>
<th>DEA</th>
<th>MTT</th>
<th>Cisplatin (nM) CA</th>
<th>DEA</th>
<th>MTT</th>
<th>Melphalan (nM) CA</th>
<th>DEA</th>
<th>MTT</th>
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<tr>
<td>V79</td>
<td>40</td>
<td>64</td>
<td>60</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>300</td>
<td>500</td>
<td>500</td>
<td>2000</td>
<td>1650</td>
<td>1100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H460</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>1.5</td>
<td>1.5</td>
<td>150</td>
<td>200</td>
<td>400</td>
<td>250</td>
<td>500</td>
<td>450</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI-H249</td>
<td>38</td>
<td>120</td>
<td>100</td>
<td>6.4</td>
<td>12.4</td>
<td>14.4</td>
<td>250</td>
<td>2000</td>
<td>2000</td>
<td>2100</td>
<td>1500</td>
<td>2750</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* CA, clonogenic assay; DEA, dye exclusion assay.
MTT ASSAY: CHEMOSENSITIVITY

Fig. 5. Dose-response curves using three assay methods following continuous exposure to drugs. A, response of V79 cells to continuous Adriamycin exposure; B, response of NCI-H460 cells to continuous cisplatin exposure; C, response of NCI-H249 cells to continuous vinblastine exposure.

assessment of chemosensitivity following a 1-h drug exposure to Adriamycin, vinblastine, and cisplatin. The first experiment was performed in V79 cells and the results are illustrated in Fig. 6. As can be seen, the assays correlated extremely well for Adriamycin and cisplatin, although the MTT assay was markedly more sensitive to vinblastine. Further studies were then performed using Adriamycin against the 2 human lung cancer cell lines, showing good correlation between the clonogenic and MTT assays, as shown in Fig. 7. Increased sensitivity to vinblastine in the MTT assay was also seen using NCI-H460 cells as illustrated in Fig. 7.

Potential Limitations of the MTT Assay. The MTT assay is a growth assay and, therefore, does not distinguish between cytostatic and cytocidal effects. The effect of cytostatic agents on the MTT assay was assessed with the use of isoleucine-depleted culture medium, which is known to block cells in cell cycle, causing a G1 arrest (28, 29). V79 cells were incubated in isoleucine-free medium for 15 h, following which isoleucine was added and cells were allowed to proliferate for a further 48 h. The MTT assay was then performed and the results were compared with those for control cells which had been grown in complete medium throughout. Significant impairment of growth was observed in isoleucine-depleted cells. In contrast, 15 h of isoleucine depletion did not affect the results of a clonogenic assay.

The effect of strong reducing agents on the interpretation of the MTT assay was also studied. Cysteamine (0.1, 1.0, and 10 mM) was added to individual wells together with MTT. Rapid reduction of MTT was observed in the solution, although no crystals were formed. Significant absorption was observed at 540 nm, although following aspiration and resolubilization in DMSO, no absorption was detectable at this wavelength. When a similar experiment was performed using V79 cells, cysteamine reduction did not affect the cellular reduction of MTT, and following resolubilization absorbance values between V79 controls and cysteamine-treated cells were similar.

Many compounds, such as Adriamycin, are known to exhibit absorption peaks in the 500–600-nm range, although aspiration of drug-containing medium from the wells resulted in no effect on absorbance readings. However, these factors can cause problems with floating cell lines, because all the medium is not removed prior to solubilization of the dye. Alteration of the oxidation-reduction balance of the cell by preincubation with agents that deplete intracellular glutathione levels, such as N-ethylmaleimide, diamide, and buthionine sulfoximine, did not affect the cellular reduction of MTT.

DISCUSSION

This study confirms, as shown in Fig. 2, that the MTT assay shows good correlation between spectrophotometric absorbance and cell number as described previously (19). Using this method, estimated doubling times for all three cell lines were quantitatively similar to those obtained using standard techniques. However, using the published method for the MTT assay (19), considerable difficulty was encountered in resolubilizing the formazan crystals. An absorbance spectrum of formazan solubilized by acid isopropyl alcohol showed a wide indistinct peak, which is greatly inferior to the absorbance peaks observed using mineral oil or DMSO as solvents. Greater solubility and absorbance readings were seen using the latter 2 solvents. Optimal results were achieved by measuring formazan crystals solubilized in mineral oil at a wavelength of 570 nm and DMSO-solubilized crystals at 540 nm.

It should be stressed that with the MTT assay, optimal conditions should be elucidated for each cell line, in terms of both cell number plated and assay duration. It is essential that sufficient time is allowed for cell death and loss of dehydrogenase activity to occur and that control cells are in exponential growth at the time the assay is processed. Because the ID_{50} is
defined as 50% reduction in absorbance compared to control values, if control cells are allowed to plateau, but treated cells continue to grow, the ID_{50} for that drug could be seriously overestimated. Likewise, with treated cells time should be allowed for cell death and loss of dehydrogenase activity; otherwise overestimation of ID_{50}s may occur. Similar conditions apply to the dye exclusion assay, where optimal seeding concentrations and assay duration are important factors in obtaining satisfactory results.

Using the MTT and dye exclusion assays, good correlation with the clonogenic assay was achieved in the chemosensitivity testing of a range of cytotoxic drugs. Differences in relative chemosensitivity between cell lines were maintained. For continuous drug exposure both nonclonogenic assays exhibited similar dose-response curves, whereas clonogenic assays appeared more sensitive. However, stronger correlation between assays was observed using 1-h drug exposures, although differences are noted for certain drugs as exemplified by the vinblastine data. This drug has cytostatic in addition to cytotoxic properties, and as a result more activity is observed with the MTT assay. It should be stressed that this assay does not differentiate between cytostatic and cytotoxic effects on cells. As has been reported previously (20), when medium containing serum or phenol red is used in this assay, high background values are observed. However, maximal absorbance for many human tumor cell lines is approximately 1.0 absorbance unit, limiting the range of detectability of this assay. Therefore, the MTT assay is limited to the detection of 1 log of cell kill, as is the clonogenic assay, in contrast, offers an excellent opportunity for the rapid testing of floating cell cultures using this technique.

Agents which alter the oxidation-reduction balance of the cell by glutathione depletion did not affect the results of the assay. Likewise, strong reducing agents cause reduction of MTT in solution but do not affect the interpretation of the assay when all the supernatant is removed prior to solubilization of the MTT formazan crystals. For these reasons difficulties remain in the handling of floating cell cultures using this technique.

In these studies cell lines with vastly different growth characteristics were used. Doubling times varied from 10 to 85 h, and although the majority of cells grew as monolayers, the small cell lung cancer line grew as floating aggregates. For chemosensitivity assays utilizing nonclonogenic techniques, there are many potential factors that may interfere with the interpretation of any results. Theoretically, for cell lines of differing growth rates, the relative contributions of in situ cell death, cell cycle delay, and inhibition of proliferation could be different and could explain observed differences in chemosensitivity seen in the continuous drug exposure experiments. However, the experiments utilizing 1-h incubation times shows these factors to be relatively unimportant, with drug exposure time being the most influential variable. Optimal assay duration for human lines should be a minimum of 4 days to allow for cell death and loss of dehydrogenase activity with a maximum of 7 days to obviate the necessity of refeeding of cultures.

It is obviously appreciated that drug exposure, as measured by area under the curve calculations, varied between assays when continuous drug exposure was used. The claim is not made that similar drug concentrations are effective in the MTT assay but that similar dose-response curves are observed. Likewise, differences in sensitivity between cell lines are detectable and are preserved to a similar degree. Accepting the limitations described, the semiautomated MTT assay offers a valid, rapid, and simple method to assess chemosensitivity in cell lines. However, the value of this assay in the testing of primary tumor samples may be limited because normal cell contaminants may also reduce the dye.

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