[CANCER RESEARCH 48, 589-601, February 1, 1988]

Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay[1]

Michael C. Alley,[2] Dominic A. Scudiero, Anne Monks, Miriam L. Hursey, Maciej J. Czerwinski, Donald L. Fine, Betty J. Abbott, Joseph G. Mayo, Robert H. Shoemaker, and Michael R. Boyd

Program Resources, Inc., National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701 [M. C. A., D. A. S., A. M., M. L. H., M. J. C., D. L. F.] and Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892 [B. J. A., J. G. M., R. H. S., M. R. B.]

ABSTRACT

For the past 30 years strategies for the preclinical discovery and development of potential anticancer agents have been based largely upon the testing of agents in mice bearing transplantable leukemias and solid tumors derived from a limited number of murine as well as human sources. The feasibility of implementing an alternate approach, namely combined *in vitro/in vivo* screening for selective cytotoxicity among panels of human tumor cell lines derived from a broad spectrum of human solid tumors is under investigation. A group of 30 cell lines acquired from a variety of sources and representing 8 lung cancer pathologies as well as 76 cell lines representing 10 other categories of human cancer (carcinomas of colon, breast, kidney, prostate, ovary, head and neck; glioma; leukemia; melanoma; and sarcoma) have exhibited acceptable growth characteristics and suitable colorimetric profiles in a single, standard culture medium. Measurements of *in vitro* growth in microculture wells by cell-mediated reduction of tetrazolium showed excellent correlation ($0.89 < r^2 < 0.98$) with measurements of cellular protein in adherent cell line cultures as well as viable cell count in suspension cell line cultures ($0.94 < r^2 < 0.99$). Since the microculture tetrazolium assay provides sensitive and reproducible indices of growth as well as drug sensitivity in individual cell lines over the course of multiple passages and several months' cultivation, it appears suitable for initial-stage *in vitro* drug screening.

INTRODUCTION

A new anticancer drug screening program based upon the use of multiple panels of human solid tumor cell lines is under development by the U. S. National Cancer Institute's Developmental Therapeutics Program, Division of Cancer Treatment (1–7). The goal of the new program is to evaluate experimental agents against groups of cell line panels each representing a major clinical category of human malignancy. Each panel (*e.g.*, lung, colon, melanoma, renal, ovarian, and central nervous system) is to contain multiple, representative human tumor cell lines. Agents showing differential or selective patterns of *in vitro* growth inhibition will be evaluated subsequently in athymic mice bearing the same human tumor cell lines found sensitive *in vitro*. This *in vitro/in vivo* approach differs from previous *in vivo* screening programs (8, 9), which most recently consisted of a murine leukemia prescreen followed by a battery of tests including several murine tumor models and three human tumor xenografts, in two fundamental ways: (*a*) a single *in vivo* murine leukemia prescreen step replaced by broad-based *in vitro* evaluations among a wide variety of cell lines; and (*b*) the major clinical forms of human solid tumors represented by panels of multiple well-characterized human tumor cell lines. In addition, the proposed *in vitro* component differs from other *in vitro* screening methodologies, *e.g.*, the human tumor colony formation assay (10, 11) which is limited by its labor-intensive nature and the more limited range of tumor types amenable to soft-agar culture.

To determine whether such an *in vitro/in vivo* disease-oriented screening program is feasible, we have examined a number of technical questions which are fundamental to *in vitro* assay systems. In this report we have assessed whether it is possible to cultivate a multiplicity of human tumor cell lines under similar culture and assay conditions; whether a colorimetric assay (*e.g.*, 12, 13) is suitable for the measurement of cell line growth inhibition; and whether individual cell lines exhibit stable and reproducible drug sensitivity profiles over time.

MATERIALS AND METHODS

Cell Line Expansion, Cryopreservation, and Characterization

Thus far, more than 100 tumor cell lines representing many human solid tumor types have been acquired from several sources following various methods of isolation and cultivation. Individual cell lines were initially photographed, expanded (two passages maximum) and cryopreserved (*master stocks*) with growth medium and split-ratios recommended by their respective sources. Only cell lines documented to be free of adventitious bacteria and pathogenic viruses (NCI-FCRF[3] Diagnostic Microbiology Lab and Animal Health Diagnostic Lab) were accepted for subsequent characterization. Following recovery of master stocks, cell lines were adapted to a single, standard culture medium: RPMI 1640 (Quality Biologicals, Inc., Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (Sterile Systems Hyclone, Logan, UT) and 2 mM L-glutamine (NCI-FCRF Central Medium Laboratory) without antibiotics and cultured under conventional culture conditions, that is, 37°C, 5% CO_2, 95% air, 100% relative humidity. Cell lines were then expanded (five passages maximum) and cryopreserved for generation of seed stocks. Established adherent cell monolayers approaching 80% confluency were harvested with trypsin/EDTA (NCI-FCRF Central Medium Laboratory) whereas some early passage adherent cell lines were harvested with solution A and 2×-crystalized trypsin III (Sigma Chemical Co.) according to the protocol of Shipley and Ham (14). Leukemia cell lines were subcultured by trituration and dilution. Small cell lung carcinoma cell lines (which generally form large aggregates in suspension under conventional culture conditions) were cultured and assayed in suspension as well as adherent monolayers utilizing poly-L-lysine pretreatment of culture vessels (15). Following recovery of seed stock, cell lines were subjected to isoenzyme analysis as well as preliminary growth and drug sensitivity assays using one or more *in vitro* growth inhibition assays (described below). Cell lines meeting basic quality assurance criteria (mycoplasma-

Received 2/2/87; revised 6/18/87, 10/6/87; accepted 11/2/87.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by National Cancer Institute contract NO1-CO-23910 with Program Resources, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services nor does mention of trade names, commercial products, or organizations imply endorsement by the U. S. Government.

[2] To whom requests for reprints should be addressed at: PDRG, Developmental Therapeutics Program, National Cancer Institute-Frederick Cancer Research Facility, Building 560, Room 32-60, Frederick, MD 21701.

[3] The abbreviations used are: NCI-FCRF, National Cancer Institute-Frederick Cancer Research Facility; DMSO, dimethyl sulfoxide; INT, 2-(*p*-iodonitrophenyl)-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride; MCPA, microculture cellular protein assay; MTA, microculture tetrazolium assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBT, 2,2'-di-*p*-nitrophenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; IC_{50}, 50% of control growth absorbance.

negative, MAP-negative, human isoenzymes only) and exhibiting suitable growth profiles were expanded (five serial passages from seed stock, maximum) and cryopreserved as a large number of aliquots designated working seed stock. Cell cryopreservation was achieved using a CryoMed controller (Model 801) and a CryoMed freezing chamber (No. 2700) with a step rate of −1°C/min followed by storage in vapor-phase liquid N_2 (NCI-FCRF Central Repository). Cell line seed stocks were tested also for *in vivo* tumorigenicity (s.c. and i.p. inoculations) in accordance with established protocols (16). Cell lines recovered from working seed stocks were subjected to repeat mycoplasma tests and to more extensive *in vitro* growth characterization. Cell lines subsequently were evaluated with respect to stability in drug sensitivity profiles over the course of 20 weekly passages. In addition, each cell line was expanded (eight passages, maximum from seed stock thaw) and cryopreserved as a large number of aliquots ("roller bottle" stock) for *in vivo* characterization and assay development.

Reagents

Tetrazolium/formazan reagents were purchased from Sigma Chemical Co. (St. Louis, MO): MTT (M2128), MTT formazan (M2003), INT (I8377), INT formazan (I7375), and NBT (N6876). DMSO was purchased from Sigma Chemical Co. (D5879), J. T. Baker Chemical Co. (9194-3, Phillipsburg, NJ), and American Burdick and Jackson Laboratories (Spectrophotometric Grade Product 081, Muskegan, MI). These chemicals were stored in unopened bottles at room temperature in the dark or in 50-ml sterile plastic tubes at −20°C in the dark. Anhydrous isopropanol (505-7) and propylene glycol (P-1009) were purchased from Sigma Chemical Co. Reagent grade hydrochloric acid (A-744) and hexane (H-302-1) were purchased from Fisher Scientific Co. Dimethylformamide (27,054-7) was purchased from Aldrich Chemical Co. (Milwaukee, WI) Propanol (spectrophotometric grade 9068-1) was purchased from American Burdick and Jackson Laboratories.

All chemotherapeutic agents were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. Crystalline stock materials were stored at −20°C. Solvent-reconstituted chemotherapeutic agents were prepared at high concentration, partitioned into multiple aliquots, and stored at −70°C. Just prior to culture application the contents of frozen vials were thawed and mixed. Measured aliquots (20–200 µl) were transferred by micropipet (Gilson Pipetman, Models P200 and P1000) equipped with polypropylene tips to standard culture medium within polypropylene tubes (Sarstedt 62.554/002 or Falcon 2098) and serially diluted in culture medium containing an appropriate concentration of vehicle.

In Vitro Growth/Growth Inhibition Assays

Microculture Tetrazolium Assay. The methodology described below represents a modification of the original MTT colorimetric assay described by Mosmann (12): In principle, the viable cell number/well is directly proportional to the production of formazan, which following solubilization, can be measured spectrophotometrically. Our modification of the original *in vitro* assay procedures has been previously described (6, 17). In brief, cells were harvested from exponential-phase maintenance cultures (T-75 cm^2 flasks; Falcon Plastics 3023), counted by trypan blue exclusion, and dispensed within replicate 96-well culture plates (Falcon Plastics 3075) in 100-µl volumes using a repeating pipet (Eppendorf repeater 4780) or multichannel pipet (Flow Labs, Titertek). Following a 24-h incubation at 37°C, 5% CO_2, 100% relative humidity (Heraeus B5060EKO$_2$ incubators or NAPCO 5300 incubators), 100 µl of culture medium, culture medium containing drug or culture medium containing drug vehicle was dispensed within appropriate wells (vehicle control group, $N = 6$; each drug treatment group, $N = 3$). Peripheral wells of each plate (lacking cells) were utilized for drug blank ($N = 2$) and medium/tetrazolium reagent blank ($N = 6$) "background" determinations. Culture plates were then incubated for 1 to 11 days prior to the addition of tetrazolium reagent. MTT stock solution was prepared as follows: 5 mg MTT/ml PBS (Quality Biologicals, Inc.) was sterile filtered with 0.45-µm filter units (Nalgene type SCN) and stored at 4°C for a maximum of 1 month. MTT working solution was prepared just prior to culture application by diluting MTT stock solution 1:5 (v/v) in prewarmed standard culture medium. Alternatively, other tetrazolium reagents (namely, INT or NBT) were prepared and utilized in a similar fashion for selected experiments. Under standard MTA conditions 50 µl of MTT working solution was added to each culture well (resulting in 50 µg MTT/250 µl total medium volume) and cultures were incubated at 37°C for 4 to 24 h depending upon individual cell line requirements. Following incubation cell monolayers and formazan were inspected microscopically: Culture plates containing suspension lines or any detached cells were centrifuged at low speed for 5 min. All but 10–20 µl of culture medium supernatant was removed from wells by slow aspiration through a blunt 18-gauge needle and replaced with 150 µl of DMSO (Burdick & Jackson) using a multichannel pipet. Following thorough formazan solubilization (trituration by pipet or vibration on a plate shaker), the absorbance of each well was measured using a microculture plate reader (Dynatech MR600; Alexandria, VA) at 540 nm (single wavelength, calibration factor = 1.00) interfaced with an Apple IIe computer. Subsequently, data were stored and analyzed through use of Apple Soft, Apple Turnover, and Lotus Symphony software. Cell line growth and growth inhibition were expressed in terms of mean (±1 SD) absorbance units and/or percentage of control absorbance (±1 SD%) following subtraction of mean "background" absorbance. Linearity and reproducibility of instrument measurements were verified by the use of formazan reagents in appropriate solvent systems.

Absorption spectra of formazan reagents as well as cell-generated formazans were measured with a UV/visible scanning spectrophotometer (Perkin-Elmer Lambda V; Perkin-Elmer Corp., Norwalk, CT). Samples were placed in 1-cm pathlength disposable polystyrene cuvets (Fisher Scientific Co. 14-385-942) except those solubilized in dimethylformamide solvent which were evaluated in 1-cm pathlength glass spectrophotometer cells (Coleman S7300-4). Freshly prepared material was analyzed in dual beam mode with 2-nm slit width, at 120 nm/min, 0.02 A threshold, and 0.5 s response. Instrument wavelength calibration was verified by examination of deuterium emission spectra to be 653.1 ± 0.3 nm.

Microculture Cellular Protein Assay. A cellular protein assay previously described by Finlay, Baguley, and Wilson (13) was adapted to the measurement of cell line growth under the same culture conditions described above for the MTA. Following 1–11 days' incubation, supernatant culture medium was removed and 200 µl of methylene blue (Sigma MB-1) solution [5 g/liter in ethanol:water (50%, v/v)] was added without delay. Following incubation at room temperature for 45 min, unbound stain was removed by plate inversion on absorbant paper and subsequent emersion/dilution in four, 1-liter washes with distilled deionized water. Bound protein stain was solubilized by the addition of 100 µl SDS (Sigma L4509) solution (1%, v/v in water) to each well. Absorbances of wells were measured at 630 nm (single wavelength, calibration factor = 1.00) using equipment and computerized analysis procedures described above for the MTA.

RESULTS

Cell Line Acquisition, Adaptation, and Cryopreservation. A key question concerning the use of *in vitro* cell lines for comparative drug evaluation has been whether a wide variety of cell lines would exhibit stable growth and drug sensitivity profiles over serial passage. To examine this question at a practical level we have evaluated the performance of multiple cell lines which represent common human solid tumor malignancies.

To date 111 cell lines derived from 10 major categories of human cancer (carcinomas of lung, colon, kidney, ovary, prostate, and head and neck; glioma; leukemia; melanoma; and sarcoma) isolated by a variety of *in vivo* as well as *in vitro* techniques have been acquired, cultivated, cryopreserved, and tested. With the exception of two early passage colon adenocarcinoma lines and one lung adenosquamous line which exhibit doubling times exceeding 120 h in our standard culture medium

formulation (each of which also proliferates slowly in their respective recommended culture medium) all tumor cell lines tested to date show suitable growth under maintenance (T-75 cm^2 flask) as well as assay conditions (96-well plate). In addition, two fibroblast cell lines (which exhibit acceptable rates of growth) metabolized MTT at levels which are adequate for detection but which may not be desirable for screening (<0.500 absorbance units/confluent monolayer). A total of 106 cell lines which meet basic quality assurance criteria and which exhibit suitable growth and colorimetric profiles are listed in Table 1. Despite the fact that these cell lines have been cultivated under a variety of conditions (including 31 different culture medium formulations) in other laboratories, each cell line appears to have adapted adequately to one set of culture conditions (and a single, standard culture medium) as indicated by exponential or near-exponential growth following inoculation at reasonable cell densities (<10,000 cells/well).

Microculture Tetrazolium Assay. Preliminary experiments using the original MTT colorimetric assay (6) revealed an apparently inadequate level of formazan generation by some cell lines, limited solubility and stability of MTT formazan, and an incompatability of the acid/isopropanol solvent system with the evaluation of some synthetic agents. Microscopic inspection of plates following tetrazolium metabolism (prior to solvent addition) revealed that these problems generally were not due to the ability of cells to metabolize MTT. Some cell lines such as NCI-H460 produced copious amounts of formazan much of which was insoluble in acid/isopropanol; other cell lines such as NCI-H322 and P388 exhibited significant formazan microscopically but low absorbance readings, again due to limited solubility of cell-generated formazan.

These findings prompted assessment of other formazan solvent systems. Solubility testing and spectral analysis with a number of solvents including DMF, DMSO, hexane, and propylene glycol showed that DMSO was the most suitable solvent for culture-generated MTT formazan as well as INT formazan; NBT formazan was not appreciably soluble in any of these neat solvents at room temperature. Microscopic inspection of cell culture plates revealed that formazans are rapidly mobilized by DMSO from sites within thick cell layers. Spectrophotometric analyzes indicate that the resulting DMSO/formazan solutions are stable and exhibit prominent absorbance in the visible light region (Fig. 1*B*). While MTT formazan reagent is in fact totally soluble in anhydrous isopropanol at a concentration of 5 mg/ml, in the presence of 0.04 N HCl/isopropanol as specified by the original procedure (12), MTT formazan exhibits a very blunted absorbance at 570 nm and increased absorbance at 420 and 300 nm (see Fig. 1*D*). Color "fading" was accompanied by a rapid, irreversible shift in absorbance maximum and appeared to be a direct consequence of medium acidification. The absorbance of MTT formazan reagent in DMSO is approximately 1.3× that of formazan in neat isopropanol (Fig. 1*C*) and more than 6.2× that observed in the acid/isopropanol solvent system. While the extinction coefficient of MTT formazan reagent in dimethylformamide (E_{513} = 18,100 M^{-1} cm^{-1}) was the highest of all neat solvents tested (Fig. 1*A* and Table 2), dimethylformamide is not compatible with polystyrene culture vessels. On the grounds of improved solubility and stability of culture-generated MTT formazan in DMSO, this solvent was adopted in our current microculture tetrazolium assay.

Spectral characteristics of reagent as well as culture-generated MTT formazan depend not only upon the organic solvent but also upon the presence or absence of serum. As shown in Fig. 2*A*, the absorption spectra of culture-derived MTT formazan and MTT formazan reagent differ significantly. The former exhibits an absorbance maximum of 553 nm (half-height bandwidth of 110 nm), whereas the later exhibits an absorbance maximum of 506 nm (half-height bandwidth of 170 nm). In the presence of DMSO and low serum concentration MTT formazan reagent exhibits a shift in the absorption maximum from 506 to 553 nm and a narrowing of the half-height bandwidth to 108 nm (Fig. 2*B*), a profile consistent with that of culture-derived MTT formazan.

As shown in Fig. 2*C* the presence of 0.5 to 5.0% (v/v) serum in DMSO substantially enhances (2.3× maximum) the molar extinction coefficient of MTT formazan (E_{506} = 15,400 *versus* E_{553} = 36,300 M^{-1} cm^{-1}). Serum concentrations of 0.5 to 5.0% in 150 μl DMSO (volume of solvent employed in final step of MTA) are equivalent to 31.5–315 μg protein/culture well (since the total protein concentration of the fetal bovine serum lot was 42 mg/ml). The presence of 10 μl (or somewhat more) culture medium (containing 10% FBS) which remains following the aspiration step coupled with protein adsorbed to culture well surfaces and cell monolayers provides an adequate amount of protein (more than 42 μg) for formazan complex formation and nearly maximum extinction. DMSO rapidly solubilizes serum as well as formazan, whereas isopropanol, propanol, hexane, and dimethylformamide are incapable of solubilizing serum at concentrations exceeding 0.0625% (data not shown). While serum is highly soluble in propylene glycol (>10% v/v is achievable), this solvent does not adequately mobilize cell-generated MTT formazan. The combined solubility of serum and formazan in DMSO unlike other organic solvents appears to be responsible for the improved extraction and detection of MTT formazan generated within cultured cell systems.

The effect of serum upon formazan extinction is not unique to MTT formazan. DMSO and serum over the same range of serum concentrations also enhance extinction and narrow the absorption bandwidth of INT formazan. While the molar extinction coefficients of INT formazan in DMSO and DMSO/serum exceeds that of MTT formazan (Table 2), the rate of formazan generation by cells is more rapid for MTT than for INT. In addition, MTT formazan (blue violet) is easier to visualize than INT formazan (red) in cell culture by light microscopy.

While MTT formazan reagent in DMSO exhibits stable spectrophotometric characteristics for several days, the absorbance of MTT formazan derived from cell culture (under conditions described for MTA above) begins to change within several hours and is affected by DMSO grade and stock DMSO storage conditions. Use of spectrophotometric grades of DMSO provide stable "background" absorbance levels for up to 2 h following solvent application, whereas use of nonspectrophotometric DMSO preparations or DMSO preexposed to air are accompanied by ever-increasing levels of "background" absorbance within 15 min of solvent application.

Microculture Growth Evaluations. *In vitro* growth evaluations on candidate cell lines were performed as follows. For each cell line a range of inoculation densities (40–20,000 cells/well) and culture durations (usually 1, 2, 4, 8, and 11 days) were evaluated (*e.g.*, Fig. 3, *A* and *B*). From such data it was possible to determine which inoculation densities give rise to a detectable and linear range of absorbance readings for a given culture duration.

To assess whether the absorbance arising from MTT formazan in adherent cell line cultures reflects total cell mass/well, replicate culture plates for each of four cell lines (A549, NCI-H460, NCI-H322, and NCI-H23) were subjected to the MTA

Table 1 *Established cell lines employed for initial in vitro growth evaluations and MTA development*

Histology cell line	Reference	Institution (Source[a])	Culture medium[b]	MTA inoculation density range[c]
Non-small cell lung cancer				
Adenocarcinoma				
A549	18	National Cancer Institute (ATCC)	5	78–1,250
A549/Asc-1	–	National Cancer Institute (NCI-TB)	17	312–2,500
Calu-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	156–5,000
Calu-6	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	156–2,500
EKVX	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	156–2,500
NCI-H23	20, 21	National Cancer Institute (A. F. Gazdar)	17	312–2,500
NCI-H324	22	National Cancer Institute (A. F. Gazdar)	17	156–5,000
NCI-H522	22	National Cancer Institute (A. F. Gazdar)	17	39–5,000
Adenosquamous carcinoma				
NCI-H125	21, 22	National Cancer Institute (A. F. Gazdar)	17	312–5,000
NCI-H647	–	National Cancer Institute (A. F. Gazdar)	17	156–5,000
Squamous cell carcinoma				
NCI-H520	21–23	National Cancer Institute (A. F. Gazdar)	17	78–5,000
NCI-H226	21, 23	National Cancer Institute (A. F. Gazdar)	17	312–2,500
SK-MES-1	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	39–1,250
Bronchiolo-alveolar carcinoma				
NCI-H322	21, 24	National Cancer Institute (A. F. Gazdar)	17	156–5,000
NCI-H358	21–24	National Cancer Institute (A. F. Gazdar)	17	156–5,000
Large cell carcinoma				
A427	18	National Cancer Institute (ATCC)	3	78–1,250
AHSM	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	312–5,000
NCI-H460	21, 22	National Cancer Institute (A. F. Gazdar)	17	39–156
Mucoepidermoid carcinoma				
NCI-H292	25	National Cancer Institute (A. F. Gazdar)	17	78–1,250
SCLC[d]				
"Classic"				
NCI-H69	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	156–5,000
NCI-H128	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	1,250–10,000
NCI-H146	20, 22	National Cancer Institute (A. F. Gazdar)	17/19	312–5,000
NCI-H187	22	National Cancer Institute (A. F. Gazdar)	17/19	1,250–10,000
NCI-H249	22	National Cancer Institute (A. F. Gazdar)	17/19	156–5,000
"Variant"				
NCI-H82	22	National Cancer Institute (A. F. Gazdar)	17/19	39–2,500
NCI-H524	22	National Cancer Institute (A. F. Gazdar)	17/19	312–5,000
"Adherent"				
DMS 114	26, 27	Dartmouth Medical School (O. S. Pettengill)	24	39–2,500
DMS 187	26, 27	Dartmouth Medical School (O. S. Pettengill)	24	156–5,000
DMS 273	27	Dartmouth Medical School (O. S. Pettengill)	24	78–1,250
SHP 77	28, 29	University of Pittsburgh (E. R. Fisher)	23	156–5,000
Colon Cancer				
COLO 205	30	Denver Medical Hospital (ATCC)	17	312–5,000
DLD-1	31	Brown University (ATCC)	17	39–625
HCC 2998	–	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	16	156–2,500
HCT 116	32	Baylor College (ATCC)	14	39–625
HT-29	19	Memorial Sloan-Kettering Cancer Center (NCI-TB)	17	39–625
LoVo	33	M. D. Anderson Hospital & Tumor Institute (ATCC)	15	39–312
LS 174T	34	Northwestern University Hospital (ATCC)	5	156–5,000
MHC 1544	35	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	16	156–10,000
SW 620	36	Scott White Clinic (ATCC)	11	39–625
SW 1116	36	Scott White Clinic (ATCC)	11	156–10,000
WiDr	37	Bureau of Biologics (ATCC)	5	39–1,250
Renal cancer				
A498	18	National Cancer Institute (ATCC)	28	78–2,500
A704	18	National Cancer Institute (ATCC)	28	312–5,000
Caki-1	19	Memorial Sloan-Kettering Cancer Center (MSK)	14	78–5,000
SN12 C	38	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	17	39–2,500
SN12 K1	38	M. D. Anderson Hospital & Tumor Institute (I. J. Fidler)	17	39–2,500
UO-31	–	National Cancer Institute (W. M. Linehan)	26	156–1,250
Breast cancer				
HS 578T	39	Naval Biosciences Laboratory (ATCC)	27	312–5,000
MCF7 WT	40	Michigan Cancer Foundation (K. Cowan)	17	39–1,250
MCF7 ADR	41	National Cancer Institute (K. Cowan)	22	39–2,500
MDA-MB-231	42	M. D. Anderson Hospital & Tumor Institute (ATCC)	11	78–5,000
ZR-75-1	43, 44	National Cancer Institute (ATCC)	17	625–5,000
ZR-75-30	43, 44	National Cancer Institute (ATCC)	17	625–5,000

Table 1—*Continued*

Histology cell line	Reference	Institution (Source[a])	Culture medium[b]	MTA inoculation density range[c]
Melanoma				
Hs 294T (A101D)	45	Naval Biosciences Lab (ATCC)	25	625–5,000
LOX	–	Norsk Hydro's Institute, Norway (O. Fodstad)	17	39–625
Malme-3M	19	Memorial Sloan-Kettering Cancer Center (ATCC)	12	312–5,000
RPMI-7951	46	Roswell Park Memorial Institute (ATCC)	3	156–5,000
SK-MEL-1	47	Memorial Sloan-Kettering Cancer Center (ATCC)	3	625–5,000
SK-MEL-2	19	Memorial Sloan-Kettering Cancer Center (ATCC)	3	312–5,000
SK-MEL-5	48	Memorial Sloan-Kettering Cancer Center (ATCC)	3	312–2,500
SK-MEL-28	48	Memorial Sloan-Kettering Cancer Center (ATCC)	3	78–5,000
SK-MEL-31	48	Memorial Sloan-Kettering Cancer Center (ATCC)	4	312–5,000
Ovarian cancer				
A2780	50	National Cancer Institute (T. C. Hamilton)	17	39–2,500
A2780 CP70	50	National Cancer Institute (T. C. Hamilton)	17	78–2,500
CAOV-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	25	625–5,000
IGROV-1	49	Institute Gustave Roussy, France (J. Benard)	17	78–625
OVCAR 3	50, 51	National Cancer Institute (T. C. Hamilton)	17	156–5,000
OVCAR 4	50, 51	National Cancer Institute (T. C. Hamilton)	17	312–5,000
OVCAR 5	50, 51	National Cancer Institute (T. C. Hamilton)	17	625–5,000
OVCAR 8	–	National Cancer Institute (T. C. Hamilton)	17	78–5,000
SK-OV-3	19	Memorial Sloan-Kettering Cancer Center (ATCC)	13	625–5,000
Prostate cancer				
DU-145	52, 53	Duke University (ATCC)	3	78–2,500
LNCaP	54	Roswell Park Memorial Institute (MSK)	20	39–1,250
PC-3	55, 56	Pasadena Center for Medical Research (M. E. Kaighn)	31	78–5,000
PC-3M	57	National Cancer Institute-FCRF (M. E. Kaighn)	31	78–1,250
UMSCP-1	58	University of Michigan (H. B. Grossman)	9	78–1,250
1013 L	–	University of Minnesota (MSK)	18	1,250–5,000
Leukemia				
CCRF-CEM	59, 60	Children's Cancer Research Foundation (ATCC)	6	1,250–10,000
CCRF-SB	59, 60	Children's Cancer Research Foundation (ATCC)	6	625–10,000
HL-60	61	National Cancer Institute (NCI-TB)	21	78–2,500
K-562	62	University of Tennessee (ATCC)	17	39–156
Molt-4	63	Roswell Park Memorial Institute (ATCC)	17	312–5,000
P388	64	Southern Research Institute (NCI-TB)	17	78–1,250 (4 day)
P388/ADR-Resist	64	Southern Research Institute (NCI-TB)	17	10–312 (4 day)
RPMI 8336	65	Roswell Park Memorial Institute (ATCC)	17	156–5,000
Central nervous system cancer				
SF126	66, 67	University of California (M. L. Rosenblum)	2	78–1,250
SF295	66, 67	University of California (M. L. Rosenblum)	2	39–1,250
SF539	66, 67	University of California (M. L. Rosenblum)	2	156–10,000
SNB19	68, 69	NINCDS (P. L. Kornblith)	29	39–2,500
SNB44	68, 69	NINCDS (P. L. Kornblith)	29	156–5,000
SNB56	68, 69	NINCDS (P. L. Kornblith)	29	39–2,500
SNB75	–	NINCDS (P. L. Kornblith)	29	78–5,000
TE671	70	Children's Hospital, Los Angeles (NCI-TB)	25	156–5,000
U251	71	University of Uppsala, Sweden (NCI-TB)	30	39–5,000
Sarcoma				
A-204	18	National Cancer Institute (ATCC)	14	78–5,000
A673	18	National Cancer Institute (ATCC)	5	156–2,500
HS 913T	46	Naval Biosciences Lab (ATCC)	25	156–5,000
HT1080	72	University of Southern California (ATCC)	5	78–2,500
Te85	73	National Cancer Institute (J. S. Rhim)	10	78–5,000
Head and neck squamous cancer				
UM-SCC-14 B,C	74, 75	University of Michigan (T. E. Carey)	9	156–2,500
UM-SCC-21 A	74, 75	University of Michigan (T. E. Carey)	9	78–5,000
UM-SCC-22 B	74, 75	University of Michigan (T. E. Carey)	9	39–625
Fibroblasts				
CCD-19Lu	46	American Type Culture Collection	30	312–5,000
IMR-90	76	Institute for Medical Research (ATCC)	8	312–5,000
Mar-Bel	46	American Type Culture Collection	30	312–5,000
MCR-5	77	National Institute for Medical Research, UK (ATCC)	1	625–5,000

[a] Cell line sources if other than original investigator were as follows: ATCC, American Type Culture Collection; MSK, Memorial Sloan-Kettering Cancer Center (Walker Laboratory); and NCI-TB, NCI-Division of Cancer Treatment Tumor Bank.

[b] Culture medium formulations recommended by source were as follows: 1 = BME, 10% FBS, Hank's BSS; 2 = EMEM, 10% FBS, NEAA, L-glutamine, gentamicin; 3 = EMEM, 10% FBS, NEAA, pyruvate; 4 = EMEM, 15% FBS, NEAA, pyruvate; 5 = EMEM, 10% FBS, NEAA, Earle's BSS; 6 = EMEM, 10% FBS (modified for suspension); 7 = EMEM, 10% FBS, NEAA, pyruvate, MEM vitamins; 8 = EMEM, 10% FBS, NEAA; 9 = EMEM, 15% FBS, P/S; 10 = EMEM, 10% FBS, P/S; 11 = L 15, 10% FBS; 12 = L 15, 15% FBS; 13 = McCoy's 5A, 15% FBS; 14 = McCoy's 5A, 10% FBS; 15 = Ham's F12, 20% FBS; 16 = Ham's F12, 15% FBS, EGF, transferrin, insulin; 17 = RPMI 1640, 10% FBS; 18 = RPMI 1640, 15% FBS, P/S; 19 = RPMI 1640, hydrocortisone, insulin, transferrin, EGF, selenium; 20 = RPMI 1640, 10% FBS, 2× L-glutamine; 21 = RPMI 1640, 20% FBS; 22 = RPMI 1640, 10% FBS, doxorubicin HCl (5 μM); 23 = RPMI 1640, % FBS; 24 = Waymouth's 752/1, 10% FBS, P/S; 25 = DMEM, 10% FBS, 4.5 g/liter glucose; 26 = DMEM, 10% FBS, 4.5 g/liter glucose, HEPES [4-(2-ydroxyethyl)-1-piperazineethanesulfonic acid], P/S/fungizone/gentamicin; 27 = DMEM, 10% FBS, 4.5 g/liter glucose, insulin; 28 = DMEM, 15% FBS; 29 = DMEM/Ham's F12(50:50), 10% FBS, P/S; 30 = CRCM, 10% FBS; 31 = PFMR-4, 3% FBS.

[c] All cells were observed to meet basic quality assurance criteria, were adapted to RPMI 1640, 10% FBS (formulation 17) and were cultivated under a single set of culture conditions (see "Materials and Methods"). Column entries are inoculation densities (cells/200 μl/well) which exhibit exponential or near-exponential growth and a linear, detectable range of absorbance values (minimum > 0.050 and maximum > 0.500 units) following culture for 7 days unless otherwise noted.

[d] SCLC, small cell lung cancer; BME, basal medium (Eagle's); FBS, fetal bovine serum; BSS, balanced salt solution; EMEM, Eagle's minimum essential medium; NEAA, nonessential amino acids; EGF, epidermal growth factor; DMEM, Dulbecco's minimum essential medium; PFMR-4, Pasadena Foundation for Medical Research-4 medium; P, penicillin; S, streptomycin; CRCM, ATCC general purpose culture medium; NINCDS, National Institute of Neurological and Communicative Disorders and Stroke.

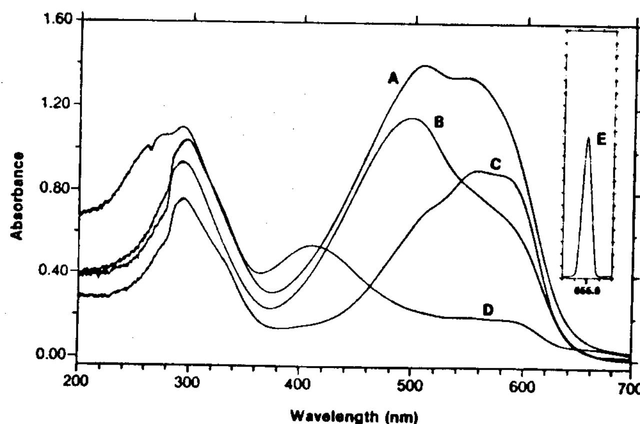

Fig. 1. Absorption spectra of MTT formazan reagent (25 μg/ml) in selected solvents. Dimethylformamide (*A*), DMSO (*B*), isopropanol (*C*), and 0.04 N HCl/isopropanol (*D*), deuterium emission spectrum (*E*).

and MCPA concurrently for multiple culture durations. Regression analyses of MTA and MCPA data for each cell line revealed overall correlation coefficients of 0.89 to 0.98 for paired data sets representing the linear and detectable regions of respective growth curves. It is important to note that while the MCPA exhibits greater sensitivity to lower cell density than does the MTA, the MCPA does not accurately measure the mass of overly confluent wells (*e.g.*, inoculation densities > 1250 cells/well on day 8, Fig. 3*B*) due to the loss of loosely attached cells during multiple washing steps (see methodology). In addition, to assess whether absorbance arising from MTT formazan in a suspension cell line reflects viable cell number/well, replicate cultures of Molt-4 were subjected to viable cell counts during the course of culture incubation. Regression analyses revealed correlation coefficients ranging from 0.94 to 0.99 for paired data in the linear, detectable absorbance and countable cell density ranges on days 1, 4, and 7 of culture.

An alternative method of plotting growth data in terms of absorbance *versus* time for each inoculation density is shown in Fig. 4. From such plots it is possible to identify when a given inoculation density exhibits exponential growth as well as when it reaches plateau-phase growth. In addition, Figs. 3 and 4 reflect the uppermost level of formazan produced (absorbance) by cells in exponential phase growth. For each cell line evaluated to date, there is a range of inoculation densities which give rise to exponential or nearly exponential growth as well as linear, detectable levels of formazan production for a given culture duration (Table 1).

Despite the increased absorbance levels noted with use of DMSO solvent in preliminary experiments with selected cell lines, subsequent experimentation with other cell lines (several fibroblasts and certain small cell lung carcinoma cell lines) revealed marginal levels of formazan production (<0.500 absorbance units) following a 4-day culture even at high inoculation density (≥20,000 cells/well). When longer culture incubations were employed (namely 7–11 days), growth generally increased to more acceptable levels (70–90% monolayer confluency). In addition, under conditions of longer tetrazolium exposure (4–8 h, and in some cases longer), marginal color development increased to more acceptable levels (>1.0 units) and assay sensitivity was improved to allow detection of less than 1,000 cells/well with >0.050 absorbance units above culture medium "background" for most cell lines.

Microculture Drug Evaluations. Preliminary *in vitro* drug evaluation data indicated (*a*) that the magnitude of measured drug sensitivity in a given cell line is primarily dependent upon culture duration and (*b*) that a range of inoculation densities give rise to optimal or near-optimal growth in a given cell line and similar drug sensitivity profiles. While 4- and 11-day culture durations were either too short or too long to achieve and maintain exponential growth in many cell lines, generally one could identify an inoculation density range for each cell line which exhibits consistent growth and drug sensitivity measurements for a 7-day culture duration. While culture durations equivalent to three cell doubling times likewise could insure exponential growth, such an approach was accompanied by widely disparate drug exposures (2.2–8 days). A series of experiments were designed to assess the impact of both inoculation density and culture duration upon drug sensitivity profiles.

In one such experiment, eight cell lines inoculated at each of three densities were exposed "continuously" to each of four drugs for each of three durations (3 days, 6 days, and three doubling times) beginning 24 h following cell inoculation. Typical examples of doxorubicin hydrochloride sensitivity profiles for two lung adenocarcinoma cell lines are shown in Fig. 5. The overall growth inhibitory concentration range observed with this drug in A549 and NCI-H23 was between 10^{-9} and 10^{-6} M (0.6–600 ng/ml) with IC_{50} values ranging from 3.3 to 45 × 10^{-9} M for A549 and from 2.1 to 96 × 10^{-9} M for NCI-H23. However, the IC_{50} ranges associated with specific culture durations were more narrow: That is, 10.4–45 × 10^{-9}, 5.2–7.6 × 10^{-9}, and 3.3–8.6 × 10^{-9} M for 4-, 5-, and 7-day cultures of A549 and 80–96 × 10^{-9}, 6.5–11.5 × 10^{-9}, and 2.1–4.1 × 10^{-9} M for 4-, 6-, and 7-day cultures of NCI-H23, respectively. Data from this and other cell line experiments demonstrated that variation in cell input over a 4-fold range has a detectable but small effect upon sensitivity to standard agents measured as percentage of control absorbance. On the other hand, increasing culture duration from 4 to 7 days (3- to 6-day drug exposure) often has a prominent effect upon drug sensitivity measurements. These data demonstrate the relative impact of inoculation density and culture duration upon drug sensitivity measurements as well as the importance of identifying a specific set of drug assay parameters based upon conditions of optimal cell growth.

Table 2 *Visible light absorption characteristics of formazan reagents in selected solvents*

Reagent	Solvent	A_{max} (nm)	Half-height bandwidth (nm)	E_{max} (cm^{-1} M^{-1})
MTT formazan	Dimethylformamide	513	172	18,100
	Propylene glycol	567	148	17,000
	Hexane	547	134	16,200
	Isopropanol	563	139	11,600
	Isopropanol/acid (0.04 N HCl)	411 (563)		6,820 (2,480)
	Dimethyl sulfoxide	506	170	15,400
	Dimethyl sulfoxide/serum (1%, v/v)	553	108	36,300
INT formazan	Hexane	496	111	17,600
	Propylene glycol	490	141	16,200
	Dimethyl sulfoxide	466	148	24,200
	Dimethyl sulfoxide/serum (1%, v/v)	648 (447)	97	48,600 (18,000)

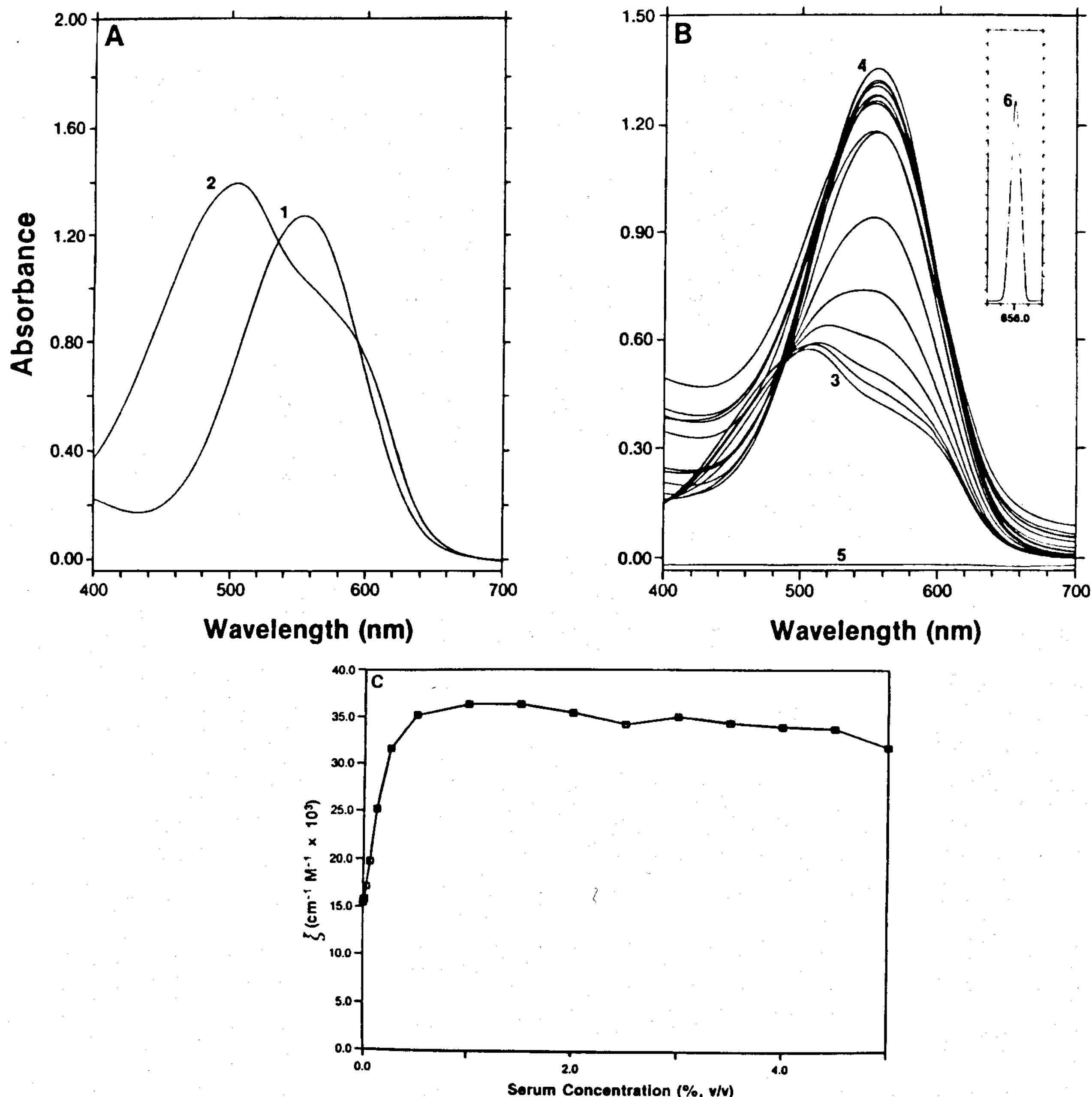

Fig. 2. In *A*, absorption spectra of (*1*) MTT formazan derived from cultured A549 cells (625 cells/well inoculation density, 4-day culture duration, 24-h MTT incubation) and (*2*) MTT formazan reagent in pure DMSO (30 μg/ml). In *B*, absorption spectra of MTT formazan reagent (12.5 μg/ml) in the absence (*3*) of serum (1% H_2O, v/v in DMSO) and in the presence (*4*) of serum (1% serum, v/v in DMSO). Absorption spectrum of 1% serum (v/v) in DMSO *vs.* pure DMSO (*5*). Deuterium emission spectrum (*6*). In *C*, effect of serum on the molar extinction coefficient of MTT formazan in DMSO.

From the standpoint of utilizing a given cell line for screening in the MTA, it is important to examine the reproducibility of drug sensitivity profiles with cryopreserved cell line stocks and aliquots of the same drug stock. In subsequent experiments each of four cell lines was evaluated under identical assay conditions on multiple occasions. As shown in Fig. 6, somewhat unique and overall reproducible profiles of cisplatin sensitivity were observed for each cell line during the course of 10–12 months and over a range of passages. Despite the testing of cisplatin at concentration intervals of ten, ranges in IC_{50} value (1.3–2.5, 2.2–3.7, 10–21, and 16.5–22.5 × 10^{-7} M for NCI-H23, LOX, A549, and MCF7, respectively) differed at most by a factor of 2.1. Data observed in this and other experiments are consistent with the effective concentration ranges reported for other *in vitro* assay systems (*e.g.*, 78–81).

Reproducibility of Drug Sensitivity Measurements. To further evaluate the suitability of the MTA for experimental drug evaluation, we have examined the reproducibility of drug sensitivity measurements during weekly testing of approximately 30 experimental compounds in each of 42 cell lines. Results for a consecutive series of assays conducted on doxorubicin HCl as a "standard" agent in each of nine "panels" of cell lines are depicted in Fig. 7. A total of 265 separate assays were performed using 42 cell lines, each one tested one or more times per week (14 lung, five colon, four CNS, five melanoma, four kidney, three ovary, two leukemia, and five other cell lines).

The reproducibility of IC_{50} values was observed to vary among cell lines: 11 cell lines exhibited less than a 2-fold range in IC_{50} value, whereas 14 cell lines exhibited greater than a 5-fold range, three of which exceeded a 10-fold range. Examination

of data and culture records indicated that deviations from the mean IC_{50} value for each cell line occurred randomly over time: no evidence of "drift" was observed for any of the lines. While some disparate IC_{50} values which occur for some cell lines (especially those with larger IC_{50} ranges) are due to technical difficulties, most could not be explained. The fact that they occur has prompted the development and inclusion of several biological and pharmacological quality assurance criteria in the performance of subsequent screening assays. Under conditions of "continuous" drug exposure and a 7-day culture duration, there is an overlapping range of sensitivities to doxorubicin HCl amongst cell lines. However, despite wide ranges in IC_{50} values for some cell lines shown in Fig. 7, it is clear that MCF7/Adr, P388/Adr, EKVX, and CCD-Lu19 are significantly more resistant than the other lines.

DISCUSSION

The feasibility of *in vitro/in vivo* drug evaluation in multiple "disease-oriented" panels of human tumor cell lines is dependent upon the successful resolution of several critical technical issues. In the current investigation, a number of key points relevant to cell culture and drug sensitivity testing were noted.

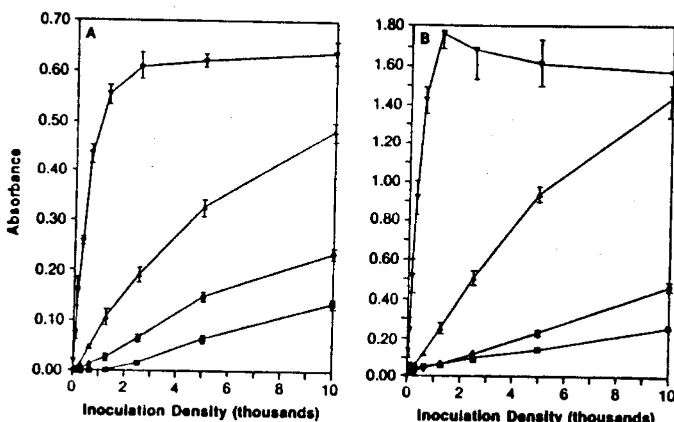

Fig. 3. Colorimetric measurement of growth in microculture plates (A549 cells): MTA (*A*) and MCPA (*B*). Culture durations: 1 day (■), 2 days (◆), 4 days (▲) and 8 days (▼). The mean ± 1 SD of three replicate wells/group is depicted.

First, cell lines derived from a broad cross-section of human solid tumors by a variety of isolation techniques could be cultivated and assayed using a single formulation of cell culture medium. Second, the majority of cell lines tested (106/111) exhibited acceptable colorimetric profiles (control growth absorbances > 0.500 units) resulting from cell-mediated reduction

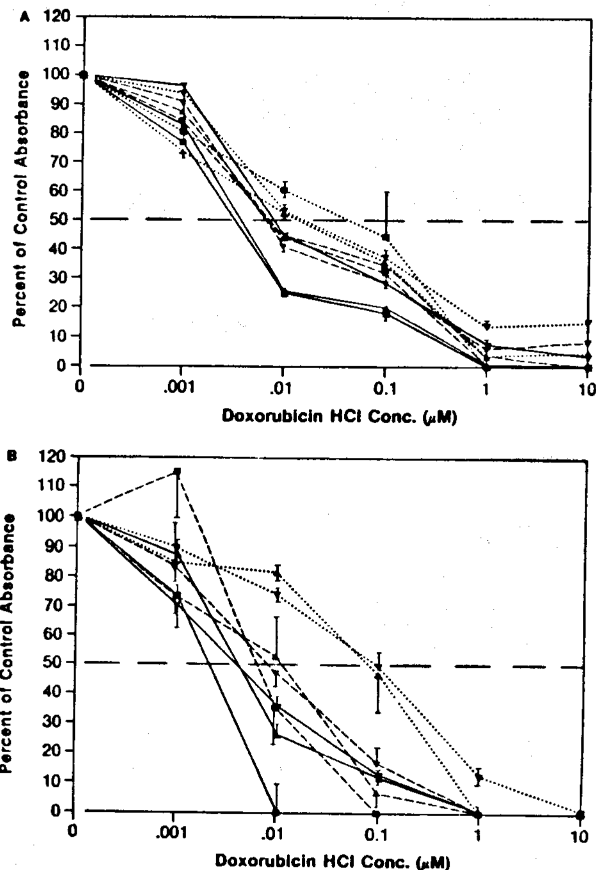

Fig. 5. Effect of inoculation density and culture duration upon drug sensitivity profiles of A549 cells (*A*) and NCI-H23 cells (*B*). Inoculation densities (cells/200 μl): 312 (■), 1250 (▲), and 5000 (▼). Culture durations: 4-day (·····), 5-, or 6-day (– – –), 7-day (——). The mean ± 1 SD of three replicate wells/group is depicted.

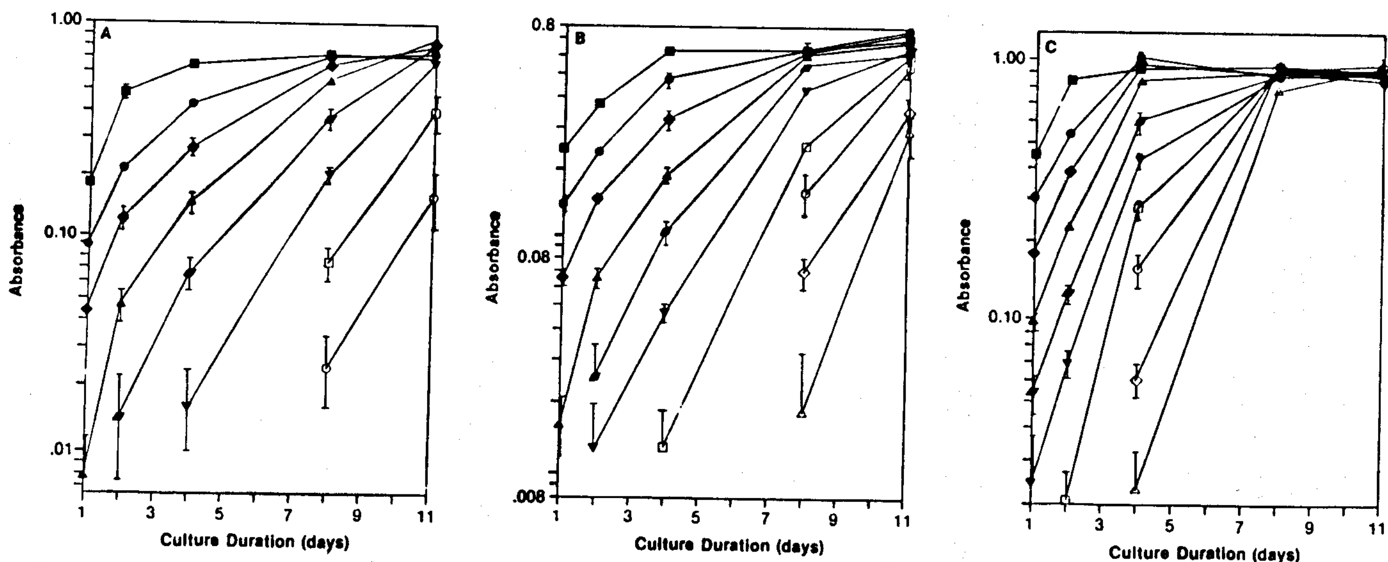

Fig. 4. Growth profile assessments using the MTA: NCI-H23 cells (*A*), A549 cells (*B*), and NCI-H460 cells (*C*). Inoculation densities (cells/200 μl): 20,000 (■), 10,000 (●), 5,000 (◆), 2,500 (▲), 1,250 (▰), 625 (▼), 312 (□), 156 (○), 78 (◇), 39 (△). The mean ± 1 SD of three replicate wells/group is depicted. Fig. 4*B* and Fig. 3*A* were derived from same data.

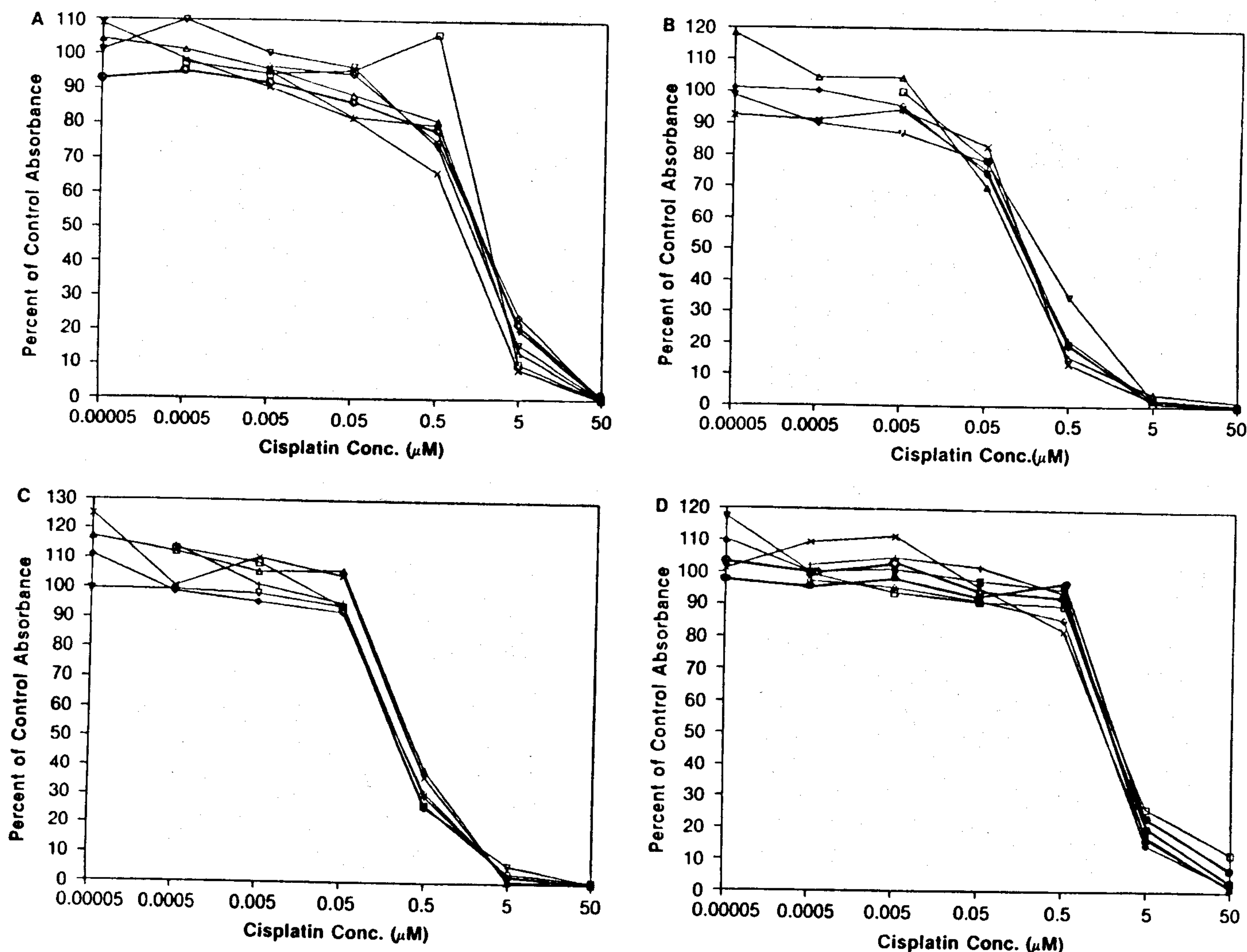

Fig. 6. Typical examples of the assessment of stability in drug sensitivity profiles. For each assay 1000 cells/well inoculation (day 1), "continuous" drug exposure (beginning day 2), and 4-h MTT incubation (day 7). *A*, A549/ASC-1 cells assayed once or twice from each of four WSS vials (P 177) at P 181, 182, 184, 185, 186, 189, and 192 (12-month interval); *B*, NCI-H23 cells following recovery from a single SS vial (P16) at P24, 32, and 40 and two WSS vials (P26) at P29, 31, and 34 (10-month interval); *C*, LOX cells following two recoveries of SS vials (P+29) at P+38, +51, and three recoveries of WSS vials (P31) at P33, 36, 39, and 44 (12-month interval); *D*, MCF 7 cells assayed at P+10 from original culture submission (P), following recovery of a MS vial (P+2) at P+4, +16, and +32 and four times following two recoveries of WSS vials (P+3) at P+7, +7, +9, and +14 (12-month interval). For clarity error bars are not shown: the SD of three replicate wells/group were generally less than 10% of the mean value.

of MTT. Third, measurements of cell growth by MTT reduction correlated well with indices of cellular protein and viable cell number. Fourth, with specific culture conditions and appropriate assay parameters the MTA was observed to provide reproducible indices of drug sensitivity in individual cell lines over the course of 10- to 12-month intervals and several passages from the thaw of multiple cryopreserved cell stocks.

In vitro drug sensitivity measurements utilizing tetrazolium reduction recently have been reported to correlate with cellular protein, dye exclusion, and clonogenic assay methodologies under a variety of culture and assay conditions (82, 83). These results coupled with our present evaluations of cell growth utilizing tetrazolium reduction, cellular protein, and viable cell count further support the view that the endpoint of tetrazolium-based assays closely approximate that of other *in vitro* assays.

It is important to note that MTA parameters employed in the current study represent one of many possible permutations for cell culture, drug assay, and data analysis and that these parameters differ somewhat from those published by other investigators in terms of the mode of drug exposures as well as culture duration. The protocol evaluated herein, involving use of "continuous" drug exposure beginning 24 h following low-density cell inoculation and a 7-day culture duration, was tentatively selected for preliminary *in vitro* drug screening on several grounds. First, many cell lines require a 7-day growth interval to achieve optimal growth and to generate levels of formazan suitable for drug assays. Second, "continuous" drug exposure insures that agents with minimal growth inhibitory activity due to limited solubility in culture medium and/or which require extended contact with cells would not be excluded prematurely from subsequent testing. In addition, while short-term *in vitro* assays (culture duration following drug treatment < six cell doubling times) may provide insufficient time to detect the total extent of "delayed death" which has been shown to occur following treatment with some chemotherapeutic agents (80), a 7-day assay would be less likely to miss such occurrences than assays of shorter duration. While the current assay format for experimental drug evaluations is seemingly sound on technical grounds, several other operational parameters also need to be investigated. For example, the applicability of a plateau-phase growth assay and/or other *in vitro* assays for a subsequent, more discriminating stage of drug evaluation

Fig. 7. Preliminary assessment of drug sensitivity reproducibility in human tumor cell line "screening panels." Overall assay conditions were as follows: 300–2000 cells/well inoculation (day 1), "continuous" exposure to doxorubicin HCl (beginning day 2), 4–8 h MTT incubation (day 7).

remains to be investigated. In addition, use of other pharmacological indices, *e.g.*, MLC, and LC_{50} (84), may provide appropriate grounds for compound selection.

Although reports concerning the original MTT colorimetric assays (12, 85) emphasized the precision and speed of the colorimetric endpoint for measuring growth stimulation, publications by others (*e.g.*, 86, 87) as well as ourselves (6, 17) have noted shortcomings in the original procedure. Nevertheless, modification of the solvent system as well as increasing culture duration and MTT incubation time has resulted in an assay methodology which provides sensitive and reproducible indices of growth as well as drug sensitivity for a large cross-section of selected human tumor and fibroblast cell lines. While the MTA and other colorimetric *in vitro* procedures have a limited range for the detection of drug activity (generally a 1 to 1.5 log range), the fact that the MTA is automatable permits a rapid means for screening of large numbers of compounds among multiple cell line panels. In addition, the finding that experimental agents can be tested under conditions of identical drug exposure meets our requirement to compare drug sensitivity profiles among disease-oriented panels of human tumor cell lines (1, 6, 7).

Even though the MTA may represent an improvement over the original MTT colorimetric assay and other *in vitro* assays in terms of performance ease, a number of its shortcomings should be indicated. First, the current methodology requires DMSO which like other organic solvents must be mixed and handled carefully as well as stored and discarded under appropriate hazard conditions. Second, tetrazolium salts can be reduced to formazans directly by a number of standard as well as experimental compounds (see below). This fact coupled with the fact that many agents exhibit absorption in the visible light region requires that each MTA plate contains corresponding "drug-blank" wells (lacking cells) as a means to determine the extent to which agents contribute to "drug-test" absorbances. Third, endpoints of the MTA appear to be somewhat susceptible to environmental factors and culture parameters. For example, it is important to include medium blanks as a means to correct control as well as test absorbances and to utilize fresh spectrophotometric grade DMSO to minimize such "backgrounds."

Use of tetrazolium salts in cell culture systems represents a relatively new application of tetrazolium/formazan technology, when compared with the chemical, biochemical, and histological applications of these substances which have been studied for many years and which have been described extensively in the literature, *e.g.*, see reviews by Nineham (88), Pearse (89), and Altman (90). While a prototype tetrazolium salt, triphenyl tetrazolium chloride, was in fact employed to assess oxidative/reductive activities of normal and malignant mammalian tissues (91) as well as the impact of selected chemotherapeutic agents on tissue metabolism (92), shortcomings of this substance (*e.g.*, slow reaction rate, lack of tissue localization, and susceptibility to reoxidation) led to its replacement by better reagents, namely INT, NBT, and MTT (90). Tetrazolium salts were later employed to facilitate detection of colonies as well as to discriminate between viable and nonviable cell groups in drug-treated tumor cell cultures (*e.g.*, 93–98).

In the current study DMSO in combination with serum was observed to improve extraction and spectrophotometric detection of cell-generated MTT formazan. In fact, the molar extinction coefficient for MTT formazan in DMSO and DMSO/serum (15,400 and 36,300 M^{-1} cm^{-1}, respectively) observed in the current experimentation approximate that for reagent as well as tissue-derived MTT formazan in dimethylformamide and dimethylformamide/cobalt (17,400 and 37,200 M^{-1} cm^{-1}, respectively) reported in the literature (99). It is also of interest that DMSO in combination with heat or strong base has been employed to solubilize NBT formazan generated by human granulocytes and macrophages and to enhance sensitivity of the NBT test (100, 101).

Further refinements and interpretation of tetrazolium-based assays for drug evaluations no doubt will benefit from careful attention to concepts and observations already described in the literature. For example, it is important to note that some substances (*e.g.*, ascorbic acid, sulfhydryl agents) are capable of reducing tetrazolium salts by direct chemical action, whereas other substances (*e.g.*, malonate, rotenone, amytal, and DCTFB) can block cell-mediated MTT reduction indirectly by inhibiting early steps in cellular respiration (89). With a group of 108 experimental agents selected at random from the National Cancer Institute repository, 10 agents prepared fresh and tested at their respective maximum soluble concentrations were observed to reduce MTT (unpublished data). However, following incubation under assay conditions for 7 days none of these agents (even at maximum soluble concentrations) were observed to reduce MTT. In fact, each of these reducing agents evaluated to date which was observed to inhibit cell growth was active at concentrations which do not react with MTT in a magnitude detectable colorimetrically. Nevertheless, to avoid false-negative endpoints in drug evaluation, each culture plate contains a standard configuration of drug blank wells (lacking cells) which permit visual as well as spectrophotometric detection of chemical MTT reduction as well as a means to measure absorbance contributions from chromogenic drug solutions.

In summary, the current investigation indicates that cell lines derived from a variety of sources can be cultivated under similar culture conditions, cryopreserved, and recovered for subsequent *in vitro* assays. The MTA provides reproducible indices of growth as well as drug sensitivity and appears suitable for first-stage (large-scale) drug screening in multiple "panels" of human tumor cell lines provided that assays are accompanied by appropriate quality control measures.

ACKNOWLEDGMENTS

The authors express their appreciation to each cell culture investigator for providing cell lines and culture information, to the Division of Cancer Treatment Board of Scientific Counselors and *ad hoc* Technical Review Committee members for constructive advice in program development, and to IMS computer and statistical support personnel for microcomputer software development and analyses of covariance. In addition, the authors wish to acknowledge laboratory staff, Curt Anthony, Cammi Bittner, Mike Currens, Eileen Gartland, Lynn North, Spring Randolph, Diane Reid, Jordis Rzecznik, Mike Selby, Donna Seniff, and Siobhan Tierney for their concerted effort and careful attention to details in cell line cultivation, assay development, and data analysis, and Laurie McMahon for preparation of the manuscript.

REFERENCES

1. Boyd, M. R. National Cancer Institute new drug development program. *In:* E. J. Frei and E. J Freireich (eds.), Accomplishments in Oncology, Volume 1, Chapter 1, Cancer therapy: Where do we go from here? pp. 68–76. Philadelphia: Lippincott, 1986.
2. National Cancer Institute planning to switch drug development emphasis from compound to human cancer-oriented strategy. Cancer Lett., *10:* 1–3, 1984.
3. Division of Cancer Treatment Board approves new screening program, natural products concepts. Cancer Lett., *11:* 4–8, 1985.
4. Boyd, M. R., Shoemaker, R. H., McLemore, T. L., Johnston, M. R., Alley, M. C., Scudiero, D. A., Monks, A., Fine, D. L., Mayo, J. G., and Chabner, B. A. New Drug Development. *In:* J. A. Roth, J. C. Ruckdescher, and T. H. Weisenburger (eds.), Thoracic Oncology, Chap. 51. New York: W. B. Saunders Co., in press.
5. Workshop on "Disease-oriented antitumor drug discovery and development", NIH, Bethesda, MD, Jan 9–10, 1985. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.
6. *Ad Hoc* review committee proceedings for National Cancer Institute *In Vitro/In Vivo* Disease Oriented Screening Project. NIH, Bethesda, MD, Sept. 23–24, 1985. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.
7. *Ad Hoc* review committee proceedings for National Cancer Institute *In Vitro/In Vivo* Disease Oriented Screening Project. NIH, Bethesda, MD, Dec. 8–9, 1986. Sponsored by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute.
8. Frei, E. The national chemotherapy program. Science (Wash. DC), *217:* 600–606, 1982.
9. Venditti, J. M. The National Cancer Institute antitumor drug discovery program, current and future perspectives: a commentary. Cancer Treat. Rep., *67:* 767–772, 1983.
10. Salmon, S. E., Hamberger, A. W., Soehnlen, B., Durie, B. G., Alberts, D. S., and Mood, T. E. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. N. Engl. J. Med., *298:* 1321–1327, 1978.
11. Shoemaker, R. H., Wolpert-DeFilippes, M. K., Kern, D. H., Lieber, M. M., Makuch, R. W., Melnick, N. R., Miller, W. T., Salmon, S. E., Simon, R. M., Venditti, J. M., and Von Hoff, D. Application of a human tumor colony-forming assay to new drug screening. Cancer Res., *45:* 2145–2153, 1985.
12. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Meth., *65:* 55–63, 1983.
13. Finlay, G. J., Baguley, B. L., and Wilson, W. R. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. Anal. Biochem., *139:* 272–277, 1984.
14. Shipley, G. D., and Ham, R. G. Improved medium and culture conditions for clonal growth with minimal serum proteins and for enhanced serum-free survival of Swiss 3T3 cells. In Vitro (Rockville), *17:* 656–669, 1981.
15. Epstein, S. L., and Lunney, J. K. A cell surface ELISA in the mouse using only poly-L-lysine as cell fixative. J. Immunol. Meth., *76:* 63–72, 1985.
16. In Vivo Cancer Models. NIH Publication No. 84-2635. Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, 1984.
17. Alley, M. C., Scudiero, D. A., Monks, A., Czerwinski, M. J., Shoemaker, R. H., and Boyd, M. R. Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines. Proc. Am. Assoc. Cancer Res., *27:* 389, March, 1986.
18. Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H., and Parks, W. P. *In vitro* cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst., *51:* 1417–1423, 1973.
19. Fogh, J., and Trempe, G. "New human tumor cell lines", Chap. 5 *In:* J. Fogh (ed.) Human Tumor Cells In Vitro, pp. 115–159. New York: Plenum Publishing Corp., 1975.
20. Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baylin, S. B., Bunn, Jr., P. A., Guccion, J. G., and Minna, J. D. Establishment of continuous, clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. Cancer Res., *40:* 3502–3507, 1980.
21. Carney, D. N., Gazdar, A. F., Bepler, G., Guccion, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H., and Minna, J. D. Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res., *45:* 2913–2923, 1985.
22. Brower, M., Carney, D. N., Oie, H. K., Gazdar, A. F., and Minna, J. D. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. Cancer Res., *46:* 798–806, 1986.
23. Gazdar, A. F., and Oie, H. K. Cell culture methods for human lung cancer. Cancer Genet. Cytogenet., *19:* 5–10, 1986.
24. Falzon, M., McMahon, J. B., Gazdar, A. F., and Schuller, H. M. Preferential metabolism of *N*-nitrosodiethylamine by two cell lines derived from human pulmonary adenocarcinomas. Carcinogenesis (Lond.), *7:* 17–22, 1986.
25. Barsky, S. H., Martin, S. E., Matthews, M., Gazdar, A., and Costa, J. C. "Low grade" mucoepidermoid carcinoma of the bronchus with "high grade" biological behavior. Cancer (Phila.), *51:* 1505–1509, 1983.
26. Pettengill, O. S., Sorenson, G. D., Wurster-Hill, D. H., Curphey, T. J., Noll, W. W., Cate, C. C., and Maurer, L. H. Isolation and growth charac-

teristics of continuous cell lines from small-cell carcinoma of the lung. Cancer (Phila.), *45:* 906–918, 1980.
27. Wurster-Hill, D. H., Cannizzaro, L. A., Pettengill, O. S., Sorenson, G. D., Cate, C. C., and Maurer, L. H. Cytogenetics of small cell carcinoma of the lung. Cancer Genet. Cytogenet., *13:* 303–330, 1984.
28. Fisher, E. R., and Paulson, J. D. A new *in vitro* cell line established from human large cell variant of oat cell lung cancer. Cancer Res., *38:* 3830–3835, 1978.
29. Koros, A. M. C., Klein, E. C., Pan, S., Atchison, R. W., Lakomy, R., Bahnson, A., and Sherer, C. Stability and utility of the unique human small cell carcinoma line SHP-77. Cancer Res., *45:* 2725–2731, 1985.
30. Semple, T. U., Quinn, L. A., Woods, L. K., and Moore, G. E. Tumor and lymphoid cell lines from a patient with carcinoma of the colon for a cytotoxicity model. Cancer Res., *38:* 1345–1355, 1978.
31. Dexter, D. L., Barbosa, J. A., and Calabresi, P. *N,N*-Dimethylformamide-induced alteration of cell culture characteristics and loss of tumorigenicity in cultured human colon carcinoma cells. Cancer Res., *39:* 1020–1025, 1979.
32. Brattain, M. G., Fine, W. D., Khaled, F. M., Thompson, J., and Brattain, D. E. Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res., *41:* 1751–1756, 1981.
33. Drewinko, B., Romsdahl, M. M., Yang, L. Y., Ahearn, M. J., and Trujillo, J. M. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res., *36:* 467–475, 1976.
34. Tom, B. H., Rutzky, L. P., Jakstys, M. M., Oyasu, R., Kaye, C. I., and Kahan, B. D. Human colonic adenocarcinoma cells. I. Establishment and description of a new line. In Vitro (Rockville), *12:* 180–191, 1976.
35. Giavazzi, R., Campbell, D. E., Jessup, J. M., Cleary, K., and Fidler, I. J. Metastatic behavior of tumor cells isolated from primary and metastatic human colorectal carcinomas implanted into different sites in nude mice. Cancer Res., *46:* 1928–1933, 1986.
36. Leibovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C., and Mabry, N. D. Classification of human colorectal adenocarcinoma cell lines. Cancer Res., *36:* 4562–4569, 1976.
37. Noguchi, P., Wallace, R., Johnson, J., Earley, E. M., O'Brien, S., Ferrone, S., Pellegrino, M. A., Milstien, J., Needy, C., Browne, W., and Petricciani, J. Characterization of WiDr: A human colon carcinoma cell line. In Vitro (Rockville), *15:* 401–408, 1979.
38. Naito, S., von Eschenbach, A. C., Giavazzi, R., and Fidley, I. J. Growth and metastasis of tumor cells isolated from a human renal cell carcinoma implanted into different organs of nude mice. Cancer Res., *46:* 4109–4115, 1986.
39. Hackett, A. J., Smith, H. S., Springer, E. L., Owens, R. B., Nelson-Rees, W. A., Riggs, J. L., and Gardner, M. B. Two syngeneic cell lines from human breast tissue: the aneuploid mammary epithelial (Hs 578T) and the diploid myoepithelial (Hs 578Bst) cell lines. J. Natl. Cancer Inst., *58:* 1795–1806, 1977.
40. Soule, H. D., Vazquez, J., Long, A., Albert, S., and Brennan, M. A human cell line from a pleural effusion derived from a breast carcinoma. J. Natl. Cancer Inst., *51:* 1409–1416, 1973.
41. Cohen, J. S., Lyon, R. C., Chen, C., Faustino, P. J., Batist, G., Shoemaker, M., Rubalcaba, E., and Cowan, K. H. Differences in phosphate metabolite levels in drug-sensitive and -resistant human breast cancer cell lines determined by ^{31}P magnetic resonance spectroscopy. Cancer Res., *46:* 4087–4090, 1986.
42. Cailleau, R., Young, R., Oliye, M., and Reeves, Jr., W. J. Breast tumor cell lines from pleural effusions. J. Natl. Cancer Inst., *53:* 661–674, 1974.
43. Engel, L. W., Young, N. A., Tralka, T. S., Lippman, M. E., O'Brien, S. J., and Joyce, M. J. Establishment and characterization of three new continuous cell lines derived from human breast carcinomas. Cancer Res., *38:* 3352–3364, 1978.
44. Engel, L. W., and Young, N. A. Human breast carcinoma cells in continuous culture: a review. Cancer Res., *38:* 4327–4339, 1978.
45. Creasey, A. A., Smith, H. S., Hackett, A. J., Fukuyama, K., Epstein, W. L., and Madin, S. H. Biological properties of human melanoma cells in culture. In Vitro (Rockville), *15:* 342–350, 1979.
46. Hay, R. J., Macy, M., Corman-Weinblatt, A., Chen, T. R., and McClintock, F. M. (eds.), American Type Culture Collection Catalogue of Cell Lines and Hybridomas, 5th Ed., Rockville, MD: American Type Culture Collection, 1985.
47. Oettgen, H. F., Aoki, T., Old, L. J., Boyse, E. A., de Harven, E., and Mills, G. M. Suspension culture of a pigment-producing cell line derived from a human malignant melanoma. J. Natl. Cancer Inst., *41:* 827–843, 1968.
48. Carey, T. E., Takahashi, T., Resnick, L. A., Oettgen, H. F., and Old, L. J. Cell surface antigens of human malignant melanoma: mixed hemadsorption assays for humoral immunity to cultured autologous melanoma cells. Proc. Natl. Acad. Sci. USA, *73:* 3278–3282, 1976.
49. Benard, J., Da Silva, J., De Blois, M-C., Boyer, P., Duvillard, P., Chiric, E., and Riou, G. Characterization of a human ovarian adenocarcinoma line, IGROV1, in tissue culture and in nude mice. Cancer Res., *45:* 4970–4979, 1985.
50. Hamilton, T. C., Young, R. C., and Ozols, R. F. Experimental model systems of ovarian cancer: applications to the design and evaluation of new treatment approaches. Semin. Oncol., *11:* 285–298, 1984.
51. Ozols, R. F. Pharmacologic reversal of drug resistance in ovarian cancer. Semin. Oncol., *12*(Suppl. 4): 7–11, 1985.
52. Mickey, D. D., Stone, K. R., Wunderli, H., Mickey, G. H., Vollmer, R. T., and Paulson, D. F. Heterotransplantation of a human prostatic adenocarcinoma cell line in nude mice. Cancer Res., *37:* 4049–4058, 1977.
53. Stone, K. R., Mickey, D. D., Wunderli, H., Mickey, G. H., and Paulson, D. F. Isolation of a human prostate carcinoma cell line (DU 145). Int. J. Cancer, *21:* 274–281, 1978.
54. Horoszewicz, J. S., Leong, S. S., Chu, T. M., Wajsman, Z. L., Friedman, M., Papsidero, L., Kim, U., Chai, L. S., Kakati, S., Arya, S. K., and Sandberg, A. A. The LNCaP cell line—a new model for studies on human prostatic carcinoma. *In:* G. P. Murphy (ed.), Models for Prostate Cancer, pp. 115–132. New York: Alan R. Liss, Inc., 1980.
55. Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., and Jones, L. W. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol., *79:* 16–23, 1979.
56. Ohnuki, Y., Marnell, M. M., Babcock, M. S., Lechner, J. F., and Kaighn, M. E. Chromosomal analysis of human prostatic adenocarcinoma cell lines. Cancer Res., *40:* 524–534, 1980.
57. Kozlowski, J. M., Fidler, I. J., Campbell, D., Xu, Z., Kaighn, M. E., and Hart, I. R. Metastatic behavior of human tumor cell lines grown in the nude mouse. Cancer Res., *44:* 3522–3529, 1984.
58. Grossman, H. B., Wedemeyer, G., Ren, L., and Carey, T. E. UM-SCP-1, a new human cell line derived from a prostatic squamous cell carcinoma. Cancer Res., *44:* 4111–4117, 1984.
59. Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A., and McCarthy, R. E. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer (Phila.), *18:* 522–529, April 1965.
60. Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., and Adams, R. A. Studies on human leukemic cells *in vitro*. *In* The Proliferation and Spread of Neoplastic Cells, pp. 65–97. Baltimore: Williams & Wilkins Company, 1968.
61. Collins, S. J., Gallo, R. C., and Gallagher, R. E. Continuous growth and differentiation of human myeloid leukemic cells in suspension culture. Nature (Lond.), *270:* 347–349, 1977.
62. Lozzio, C. B., and Lozzio, B. B. Human chronic myelogenous leukemia cell-line with positive Philadelphia chromosome. Blood, *45:* 321–334, 1975.
63. Minowada, J., Ohnuma, T., and Moore, G. E. Brief communication: Rosette-forming human lymphoid cells. I. Establishment and evidence for origin of thymus-derived lymphocytes. J. Natl. Cancer Inst., *49:* 891–895, 1972.
64. Schabel, Jr., F. M., Skipper, H. E., Trader, M. W., Laster, Jr., W. R., Griswold, Jr., D. P., and Corbett, T. H. Establishment of cross-resistance profiles for new agents. Cancer Treat. Rep., *67:* 905–922, 1983.
65. Matsuoka, Y., Moore, G. E., Yagi, Y., and Pressman, D. Production of free light chains of immunoglobulin by a hematopoietic cell line derived from a patient with multiple myeloma. Proc. Soc. Exp. Biol. Med., *125:* 1246–1250, 1967.
66. Rutka, J. T., Giblin, J. R., Høifødt, H. K., Dougherty, D. V., Bell, C. W., McCulloch, J. R., Davis, R. L., Wilson, C. B., and Rosenblum, M. L. Establishment and Characterization of a cell line from a human gliosarcoma. Cancer Res., *46:* 5893–5902, 1986.
67. Rutka, J. T., Giblin, J. R., Dougherty, D. Y., Liu, H. S., McCulloch, J. R., Bell, C. W., Stern, R. S., Wilson, C. B., and Rosenblum, M. L. Establishment and characterization of five cell lines derived from human malignant gliomas. Acta Neuropathol., in press, 1988.
68. Kornblith, P. L., and Szypko, P. E. Variations in response of human brain tumors to BCNU *in vitro*. J. Neurosurg., *48:* 580–586, 1978.
69. Kornblith, P. L., Smith, B. H., and Leonard, L. A. Response of cultured human brain tumors to nitrosoureas: Correlation with clinical data. Cancer (Phila.), *47:* 255–265, 1981.
70. McAllister, R. M., Isaacs, H., Rongey, R., Peer, M., Au, W., Soukup, S. W., and Gardner, M. B. Establishment of a human medulloblastoma cell line. Int. J. Cancer, *20:* 206–212, 1977.
71. Ponten, J. Neoplastic human glia cells in culture. *In:* J. Fogh (ed.), Human Tumor Cells In Vitro, Chap. 7, pp. 175–204. New York: Plenum Publishing Corp., 1975.
72. Rasheed, S., Nelson-Rees, W. A., Toth, E. M., Arnstein, P., and Gardner, M. B. Characterization of a newly derived human sarcoma cell line (HT-1080). Cancer (Phila.), *33:* 1027–1033, 1974.
73. Rhim, J. S., Cho, H. Y., and Huebner, R. J. Non-producer human cells induced by murine sarcoma virus. Int. J. Cancer, *15:* 23–29, 1975.
74. Krause, C. J., Carey, T. E., Ott, R. W., Hurbis, C., McClatchey, K. D., and Regezi, J. A. Human squamous cell carcinoma: establishment and characterization of new permanent cell lines. Arch. Otolaryngol., *107:* 703–710, 1981.
75. Carey, T. E. Establishment of epidermoid carcinoma cell lines. *In:* R. E. Wittes (ed.), Head and Neck Cancer, pp. 287–314. New York: John Wiley & Sons, Ltd., 1985.
76. Nichols, W. W., Murphy, D. G., Cristofolo, V. J., Toji, L. H., Greene, A. E., and Dwight, S. A. Characterization of a new human diploid cell strain, IMR-90. Science (Wash. DC), *196:* 60–63, 1977.
77. Jacobs, J. P., Jones, C. M., and Baille, J. P. Characteristics of a human diploid cell designated MRC-5. Nature (Lond.), *227:* 168–170, 1970.
78. Roper, P. R., and Drewinko, B. Comparison of *in vitro* methods to determine drug-induced cell lethality. Cancer Res., *36:* 2182–2188, 1976.
79. Drewinko, B., Patchen, M., Yang, L-Y., and Barlogie, B. Differential killing efficacy of twenty antitumor drugs on proliferating and nonproliferating human tumor cells. Cancer Res., *41:* 2328–2333, 1981.
80. Weisenthal, L. M., Dill, P. L., Kurnick, N. B., and Lippman, M. E. Comparison of dye exclusion assays with a clonogenic assay in the deter-

mination of drug-induced cytotoxicity. Cancer Res., *43:* 258–264, 1983.
81. Alley, M. C., and Lieber, M. M. Drug application to the surface of soft-agarose cell cultures. *In:* S. E. Salmon and J. M. Trent (eds.), Human Tumor Cloning, pp. 205–214. New York: Grune and Stratton, Inc., 1984.
82. Finlay, G. J., Wilson, W. R., and Baguley, B. C. Comparison of *in vitro* activity of cytotoxic drugs towards human carcinoma and leukemia cell lines. Eur. J. Cancer Clin. Oncol., *22:* 655–662, 1986.
83. Carmichael, J., DeGraff, W. G., Gazdar, A. F., Minna, J. D., and Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res., *47:* 936–942, 1987.
84. Skehan, P., Thomas, J., and Friedman, S. J. Postconfluency MDCK monolayers as an *in vitro* model of solid tumor chemosensitivity. Cell Biol. Toxicol., *2:* 357–368, 1986.
85. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., and Coffman, R. L. Two types of murine helper T-cell clone I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol., *136:* 2348–2357, 1986.
86. Denizot, F., and Lang, R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Meth., *89:* 271–277, 1986.
87. Tada, H., Shiho, O., Kuroshima, K., Koyama, M., and Tsukamoto, K. An improved colorimetric assay for interleukin 2. J. Immunol. Meth., *93:* 157–165, 1986.
88. Nineham, A. W. The chemistry of formazans and tetrazolium salts. Chemistry Rev., *55:* 355–483, 1955.
89. Pearse, A. G. E. Principles of oxidoreductase histochemistry. *In:* Histochemistry, Theoretical and Applied, 3rd ed., Chap. 20. Edinburgh: Churchill Livingston, 1972.
90. Altman, F. P. Tetrazolium salts and formazans. *In:* Prog. Histochem. Cytochem. *9:* 1–56, 1977.
91. Straus, F. H., Cheronis, N. D., and Straus, E. Demonstration of reducing enzyme systems in neoplasms and living mammalian tissues by triphenyltetrazolium chloride. Science (Wash. DC), *108:* 113–115, 1948.
92. Black, M. M., and Speer, F. D. Further observations on the effects of cancer chemotherapeutic agents on the *in vitro* dehydrogenase activity of cancer tissue. J. Natl. Cancer Inst., *14:* 1147–1158, 1954.
93. Schaeffer, W. I., and Friend, K. Efficient detection of soft-agar grown colonies using a tetrazolium salt. Cancer Lett., *1:* 259–262, 1976.
94. Bol, S. Van Den Engh, G., and Visser, J. A technique for staining haemopoietic colonies in agar cultures. Exp. Hematol., *5:* 551–553, 1977.
95. Alley, M. C., Uhl, C. B., and Lieber, M. M. Improved detection of drug cytotoxicity in the soft-agar colony formation assay through use of a metabolizable tetrazolium salt. Life Sci., *31:* 3071–3078, 1982.
96. Alley, M. C., and Lieber, M. M. Improved optical density of colony enlargement and drug cytotoxicity in primary soft agar cultures of human solid tumour cells. Br. J. Cancer, *49:* 225–233, 1984.
97. Alley, M. C., Powis, G., Appel, P. L., Kooistra, K. L., and Lieber, M. M. Activation and inactivation of cancer chemotherapeutic agents by rat hepatocytes cocultured with human tumor cell lines. Cancer Res., *44:* 549–556, 1984.
98. Alley, M. C., and Lieber, M. M. Measurement of human tumour cell growth in soft-agar culture using computer-assisted volume analysis. Br. J. Cancer, *52:* 205–214, 1985.
99. Altman, F. P. Studies on the reduction of tetrazolium salts. III. The products of chemical and enzyme reduction. Histochemistry, *38:* 155–171, 1974.
100. Jarstrand, C. The NBT (nitroblue tetrazolium) activity of neutrophil granulocytes in patients with influenza A infection. Scand. J. Infect. Dis., *9:* 5–7, 1977.
101. Rook, G. A. W., Steele, J., Umar, S., and Dockrell, H. M. A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ-interferon. J. Immunol. Meth., *82:* 161–167, 1985.