Differences in myristic acid synthesis and in metabolic rate for P388 cells resistant to doxorubicin

Phillip J. Wilder, David K. Overman, Todd C. Tenenholz, and Peter L. Gutierrez[1]

University of Maryland Cancer Center, 655 West Baltimore Street, Baltimore, MD 21201

Abstract Lipids extracted from doxorubicin-resistant murine leukemia cells (P388/ADR) contained greater relative amounts of myristic and palmitoleic acids than lipids from sensitive cells (P388). This was seen in both the phospholipid and neutral lipid fractions under two nutritional conditions. Correspondingly in P388/ADR cells, myristic acid comprised a greater proportion of the products of the fatty acid synthetase system, and acyl-CoA 9-desaturase activity was transiently greater than in P388. Similar alterations in myristic acid synthesis were exhibited by DC3F/AD X, N417/VP-16, and P388/AZQ^{30}U cells but not by CH^{R}C5 or HL60/AR cells. This alteration was independent of alterations in the P180 glycoprotein and might be linked via the myristoylation of proteins to a different mechanism of drug resistance. Doxorubicin-resistant P388/ADR cells also exhibited a much higher rate of oxidative energy production.—**Wilder, P. J., D. K. Overman, T. C. Tenenholz, and P. L. Gutierrez.** Differences in myristic acid synthesis and in metabolic rate for P388 cells resistant to doxorubicin. *J. Lipid Res.* 1990. 31: 1973–1982.

Supplementary key words 9-acyl desaturase • cell cultures • fatty acid composition • fatty acid synthetase • leukemia • multidrug resistance • myristoyltransferase • neutral lipid • phospholipid • oxygen consumption

The study of drug resistance in cancer is very important, first because of its obvious consequences in the clinical setting, and second, because through the understanding of resistance, mechanism(s) of drug action can be better understood and hypotheses concerning these mechanisms can be tested. There is no unifying hypothetical mechanism by which cancer cells become resistant to antitumor agents. In some cases, a population of cells appears to have primary or intrinsic drug resistance that is selected for survival upon drug treatment (1–3). At other times, cells appear to acquire drug resistance after exposure to drugs (1). One common phenomenon in this last category is multidrug resistance (MDR). Cells that display multidrug resistance are cells that, after being exposed to a natural product such as colchicine or the antibiotic anticancer agent doxorubicin, demonstrate resistance to a variety of structurally unrelated natural products, including anticancer agents such as vincristine, vinblastine, and actinomycin D (4, 5). In mammalian cells, the MDR phenotype has been associated with a cell surface P-glycoprotein with a molecular mass in the range of 170–180 kDa (5) that is presumed to activate an "efflux pump" responsible for low drug accumulation (6, 7). Low drug accumulation leads to drug resistance.

In this study we investigate cell membrane fatty acid content and synthesis in cells resistant to doxorubicin (Adriamycin, ADR) because membranes may play a role in drug transport and thus be involved in possible mechanisms of drug resistance. Accumulated evidence indicates that nucleic acid damage may not be the only mechanism of action of quinone-containing antitumor agents such as anthracycline analogs (e.g., doxorubicin). Critical interactions occur at the membrane level and these interactions can lead to cell death. For example, doxorubicin has been shown to influence lipid organization, membrane morphology, and fluidity (8); doxorubicin can affect the phosphatidylinositol second messenger system at multiple steps (9, 10); and polymer-bound doxorubicin, which does not penetrate the cell, is cytotoxic to cells in culture (8).

Lipids constitute a very important part of membranes. However, little information has been reported regarding differences in lipids between cell lines that are sensitive or resistant to anticancer agents. In P388 murine leukemia cells resistant to doxorubicin (P388/ADR), cells appeared to have more triglyceride and sphingomyelin, but less phosphatidylcholine than drug-sensitive (P388) cells (11). The authors attributed this difference to lower phosphocholine transferase activity in P388/ADR cells. Other workers reported that there were no differences in content of neutral lipid families, phospholipids, or gangliosides betwen P388/ADR and parental P388 lines (P388) (12). Using Friend leuke-

Abbreviations: FCS, fetal calf serum; αMEM, α minimal essential media; VP-16, etoposide; MDR, multidrug resistance; HPLC, high performance liquid chromatography; TLC, thin-layer chromatography.

[1]To whom reprint requests should be addressed at: Division of Developmental Therapeutics, UMCC, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201.

mia lines with various degrees of resistance to doxorubicin, differences were reported in the ratios of phosphatidylcholine to both phosphatidylethanolamine and sphingomyelin (13). Ratios were greater in resistant lines although these ratios did not correlate with the degree of resistance. Acute lymphoblastic T cells resistant to vinblastine contained more sphingomyelin and cardiolipin and less phosphatidylethanolamine and phosphatidylserine than sensitive lines (14).

Even less information is available regarding differences in fatty acid distribution between cell lines sensitive or resistant to anticancer agents. Differences were reported in relative amounts of polyunsaturated fatty acids in doxorubicin-sensitive and -resistant rat glioblastoma cells (15). Resistant cells took up fatty acid from the media more rapidly, which could explain the differences in relative composition of fatty acids. Other workers reported no major difference in the distribution of fatty acids in phospholipid, neutral lipid, glycolipid, or gangliosides between P388/ADR and P388 cell lines (12).

In this study the fatty acid composition of doxorubicin-resistant (P388/ADR) cells is examined using P388 cells as a basic for comparison. The differences found are traced to altered synthesis of fatty acids rather than to altered rates of uptake from the media. In addition, oxidative metabolism is shown to be greater in resistant cells.

Alterations previously found in the membranes of multidrug-resistant cells, while significant, were generally specific to the cell line being studies. The major finding of this study was common to four of the six multidrug-resistant cell lines investigated.

MATERIALS AND METHODS

Radiolabeled [1-^{14}C]acetic acid (58.0 mCi/mmole, sodium salt in ethanol), [1-^{14}C]linoleic acid (55.6 mCi/mmole, in ethanol), and [1-^{14}C]palmitic acid (5.7 or 56.6 mCi/mmole, in ethanol) were obtained from New England Nuclear (Boston, MA). Doxorubicin (Adriamycin) was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, NIH (Bethesda, MD).

Cell culture

Murine leukemia cell lines P388, P388/ADR (16), and diaziquone-resistant P388/AZQ^{30}U were maintained in vitro in our laboratory by serial culture in RPMI 1640 (GIBCO, Grand Island, NY) containing 0.01 mM mercaptoethanol, 50 units/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine, and 15% (or where indicated 5%) fetal calf serum (FCS) (Biofluids, Rockville, MD). Cells were passed twice a week and maintained at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Under these conditions, the cells have a population doubling time of 14 to 18 h and achieve maximum cell densities of 2 to 4 $\times$ 10^6 cells/ml. P388/AZQ^{30}U are partial revertants of a cell line produced by exposure in vivo to increasing concentrations of diaziquone (17). Cross-resistance of these cells to doxorubicin was 4- to 5-fold compared with 24-fold in the initial resistant line (17).

Human small cell lung cancer line NCI-N417 (18) and an etoposide (VP-16)-resistant subline were generous gifts from Dr. Austin Doyle. They were grown in RPMI 1640 supplemented with 2% FCS, 100 nM hydrocortisone, 5 mg/l transferrin, 100 nM estradiol, 5 mg/l (24 IU/mg) insulin, and 5 μg/l sodium selenite. The resistant line was developed by Dr. Doyle through repeated 2-h challenges with concentrations of VP-16 up to 150 μM. The line N417/VP-16 is the product of two subcloning steps, once by limiting dilution and once by selection from a colony grown in agar. It is 4-fold resistant to VP-16 in a clonogenic assay and 3-fold cross-resistant to doxorubicin (Doyle, A., personal communication).

Chinese hamster lung cell lines DC3F and actinomycin D-resistant DC3F/AD X (19) were generous gifts from Dr. June Biedler. They were grown in 45% αMEM, 45% Hamm's F12 media, and 10% heat-denatured FCS. The resistant line was maintained in 10 μM actinomycin D.

Chinese hamster ovary lines AUX B1 and colchicine-resistant CH^{R}C5 (20) were generous gifts from Dr. Victor Ling. They were grown in αMEM with 10% heat-denatured FCS.

Human promyelocytic leukemia cell lines HL60 and doxorubicin-resistant HL60/AR (21) were generous gifts from Dr. Alex Hindenberg. They were grown in RPMI 1640 supplemented with either 10% FCS or 5 μg/ml each of insulin and transferrin (22). The resistant line did not survive long when diluted into serum-free media and had to be used within a few days.

Lipid analysis

Cells were grown for 3 days as described above, washed with 0.15M NaCl, and counted in a ZBI model Coulter counter (Coulter Electronics Inc., Hialeah, FL). Lipids were extracted from 2-4 $\times$ 10^8 pelleted cells with 20 volumes of chloroform–methanol 2:1 and filtered. Solvents were removed with a rotary evaporator and lipids were resuspended in hexane–chloroform 90:10. For separation into lipid classes, the sample (in chloroform) was applied to a 2.5 ml Biosil column (Biosil-A, 100/200, Bio-Rad, Richmond, VA) and sequentially eluted with 25 ml chloroform, 50 ml acetone, and 25 ml methanol to obtain the neutral lipid, glycolipid, and phospholipid fractions, respectively. Triacylglyceride (including any mono- and diglyceride present) was assayed by the method of Soloni (23). Lipid phosphate was assayed by the method of Ames (24).

Transesterification of lipid samples for gas–liquid chromatography was accomplished by the procedure of Morrison and Smith (25) using boron trifluoride. A Perkin-Elmer Sigma 2 gas chromatograph equipped with a Waters 740 Data Module integrator (Millipore, Milford, MA) was used to analyze the fatty acid methyl esters. The 3' $\times$ 1/8" OD glass column was packed with 10% SP-2330 on 100/120

Chromosorb (Supelco Inc., Bellefonte, PA). The oven temperature was programmed to rise from 150 to 225 °C at 6 °/min after an initial 5 min at 150 °C. The carrier gas was N_2 at a flow rate of 30 ml/min. Peaks were identified by comparison of retention times with those of known standards. To confirm the identity of some unsaturated fatty acids, samples were hydrogenated using platinum dioxide as a catalyst. H_2 was bubbled through the sample in methanol for 30 min.

Rates of fatty acid uptake

Three days after the previous passage, cells were suspended in fresh media to 3.5 or 7 × 10^5/ml. One μCi of 1-^{14}C-labeled plamitic or linoleic acid was added with the FCS to 45 ml of cells. Flasks without ^{14}C were used to determine cell concentrations. At various time points from 0 to 24 h, cells were harvested by centrifugation and washed twice with 0.15 M NaCl. The final pellet and aliquots of supernatants were counted for ^{14}C. Rates of uptake were determined for each cell line over the time intervals 0-12, 12-18, and 18-24 h (% of fatty acid taken up/h per avg cell number). A time weighted average was taken of these rates and used as the rate of uptake.

Incorporation of [1-^{14}C]acetate

This assay was performed to ascertain whether there was any difference in the synthesis of fatty acids by analyzing the amount of ^{14}C incorporated from [1-^{14}C]acetate into fatty acids. Four days after a previous passage, cells were suspended in fresh media. Five μCi of sodium [1-^{14}C]acetate was added at 0 h to flasks with 7 × 10^5 cells/ml and at 24 h to flasks started with 3.5 × 10^5 cells/ml. Cells were harvested by centrifugation 24 h after addition of [1-^{14}C]acetate and rinsed with 0.15 M NaCl. Lipids were extracted as above and hydrolyzed by refluxing for 1 h in ethanol-40% KOH 2:1, using 0.005% butylated hydroxytoluene as an antioxidant. After acidification with HCl, free fatty acids were extracted with three aliquots of hexane and washed with water. The hexane was evaporated under N_2 and free fatty acids were resuspended in methanol before analysis on HPLC. Flasks of cells without [1-^{14}C]acetate were used to determine cell concentrations at 0, 24, and 48 h. In one experiment, lipids were further separated by TLC on silica gel G plates (Analtech, Inc., Newark, DE) using petroleum ether-ethyl ether-glacial acetic acid 80:20:1.

HPLC of fatty acids

Fatty acids were separated by HPLC in order to collect fractions and correlate their identity with radioactivity. Free fatty acids were separated on a 220 × 4.6 mm ODS HPLC column (Spheri-5, Brownlee Labs, Santa Clara, CA) eluting with 82 or 91% acetonitrile, and 18 or 9% 100 mM H_3PO_4 at 1 ml/min. About 10,000 dpm were applied to the column in a volume of 0.05 ml. Column temperature was maintained at 35 °C, and the effluent was monitored at 192 or 200 nm and fractions were collected for beta scintillation counting (Beckman LS5801 scintillation counter). Samples were hydrogenated as above and rerun on HPLC to quantify ^{14}C in co-eluting fatty acids. Unsaturated fatty acids were identified by comparison of retention times with those of known standards. The retention times of saturated fatty acids were determined using [1-^{14}C]myristic, [1-^{14}C]palmitic, and hydrogenated fatty acid samples. TLC with the 80:20:1 solvent system (see above) was used to separate [^{14}C]cholesterol from pooled samples for use in determining its retention time.

O_2 consumption

Rates of O_2 consumption were obtained to investigate differences between metabolic rates of P388 and P388/ADR cells. The data were obtained using a model 53 biological oxygen monitor system (Yellow Springs Instrument Co. Inc., Yellow Springs, OH). Unless otherwise noted, cells were harvested 4 days after a previous passage and resuspended in fresh media containing 15% FCS (pH 7.4). Three ml (10^7 cells/ml) was used and the temperature was maintained at 37 °C.

Acyl-CoA 9-desaturase activity

Results from [1-^{14}C]acetate incorporation indicated proportionally higher ^{14}C levels in oleic (18:1) and palmitoleic (16:1) acids. Desaturase activity was evaluated to see whether this was due to increased enzymatic activity. Acyl-CoA 9-desaturase activity was assayed by a 4-h incubation of cells with [1-^{14}C]palmitic acid. One μCi of palmitic acid mixed with 250 μg bovine serum albumin (essentially fatty acid-free, Sigma Chemical Co., St. Louis, MO) was added to 45 ml cells. Cells were taken 4 days after a previous passage and either used directly, 1 h after dilution with fresh media to 7 × 10^5 cells/ml, or 24 h after dilution with fresh media to 3.5 × 10^5 cells/ml. At the end of the 4-h time period, cells were harvested and processed for separation of fatty acids by HPLC.

Myristoyltransferase activity

Myristoyltransferase activity was evaluated by Constance Glover in Dr. Ronald Felsted's laboratory as previously described (26) with the following modification. Pelleted cells were vortexed in homogenization buffer to which 0.5% Triton X-100 was added. The rationale for this assay was to investigate reasons for increased pools of myristic acid in doxorubicin-resistant cells.

Statistics

The two-tailed Student's *t*-test was used for statistical analysis except for tests for myristic acid in Tables 6 and 7 and for palmitoleic acid in Table 6 where the one-tailed test was used. Averages and standard deviations are reported. Paired analysis was often useful since replicate experiments were performed over several months time span, while samples from each cell line or condition were run simultaneously.

When performing paired analyses, values for drug-sensitive cells were subtracted from values for resistant cells and the difference was divided by the value for sensitive cells $\frac{(R - S)}{S}$.

RESULTS

Lipid composition

The relative amount of each fatty acid present in the neutral lipids and phospholipids of cells grown in media containing 15% FCS was determined by gas-liquid chromatography and expressed as a percentage of the total content of the seven fatty acids analyzed (**Table 1**). Other fatty acids present in small amounts were excluded to reduce accumulated error and variability in determining percentages. P388/ADR cells had greater proportions of myristic (14:0) and palmitoleic acid (16:1) than did P388 cells. Lipid phosphate and triglyceride, the major source of neutral lipid fatty acids, were assayed and expressed as μg per 10^8 cells (Table 1). P388/ADR cells has less triglyceride and slightly more phospholipid than P388 cells.

To determine the source of the differences in fatty acid composition, P388 and P388/ADR cells were grown in media containing only 5% FCS. This should reduce the supply of fatty acid from the media and increase the contribution of de novo synthesis to cellular fatty acid. The n-6 and n-3 families of fatty acids cannot be synthesized de novo in animal cells and may be used to monitor this change. Relative amounts of the n-6 fatty acids, linoleic and arachidonic, were lower in both lipid fractions for cells grown in 5% FCS (Table 1 and Table 2). The n-3 fatty acids, docosapentaenoic and docosahexaenoic, were similarly affected (data not shown). The relative amount of oleic acid (18:1) was greater in both phospholipid and neutral lipid of cells grown in 5% FCS, presumably due to a mechanism that minimizes changes in membrane fluidity (27). Cells grown in 5% FCS had less triglyceride (neutral lipid fraction) than cells grown in 15% FCS (Tables 1 and 2). The phospholipid content was reduced by 20-40% which was statistically significant only for P388 cells.

When grown in 5% FCS, P388/ADR had 40% more phospholipid than P388 cells (Table 2). In both lipid fractions the relative amounts of myristic, palmitic and palmitoleic acid were greater in P388/ADR than in P388 cells while the relative amounts of stearic and oleic acid were lower. Proportionally, these differences were greater for myristic and palmitoleic (141% and 68%, $\frac{(ADR - P388)}{P388} \times 100$; Table 2) than for the other fatty acids (−17% to 14%). These differences in the content of myristic and palmitoleic acid correspond to and are larger than the differences found for cells grown in 15% FCS.

Fatty acid uptake

Fatty acid uptake was used as a second method to ascertain that the differences in fatty acid composition were not due to differences in uptake of fatty acid from culture media. The uptake of [1-^{14}C]linoleic or [1-^{14}C]palmitic acid into P388 and P388/ADR cells was examined and relative rates of uptake were calculated as given in Materials and Methods. Relative rates of uptake were similar for these two fatty acids and no distinction will be made between them in the following results. An average of 41% (± 13 SD, n = 7) of the added 1-^{14}C-labeled fatty acid was taken up by P388 cells in a fairly linear manner over 24 h. The unrecovered radioactivity, 6% ± 5 for P388 and 8% ± 5 for P388/ADR, did not correlate with time in culture, and thus, was not thought to be due to oxidation of fatty acids. No significant difference was observed between the relative rates at which P388 and P388/ADR cells took up fatty acids (**Table 3**).

In the presence of 40 nM doxorubicin, P388 cell numbers were 19% lower after 24 h than in the absence of doxorubicin. However, rates of fatty acid uptake were unaffected (Table 3). Similarly, 2000 nM doxorubicin resulted in 16% lower cell numbers for P388/ADR cells without affecting rates of fatty acid uptake.

TABLE 1. Fatty acid distribution and lipid content of cells grown in 15% fetal calf serum

Lipid	Fatty Acid 14:0	16:0	16:1	18:0	18:1	18:2	20:4	Total Content
	% of total fatty acids							*$\mu g/10^8$ cells*
Phospholipid								
P388[a]	2.6 ± 0.8	15.1 ± 1.1	3.7 ± 0.2	22.4 ± 1.3	42.0 ± 2.0	3.4 ± 0.4	10.7 ± 0.8	1290 ± 300
P388/ADR	4.1 ± 0.9	14.7 ± 0.7	4.7 ± 0.1	22.2 ± 1.8	40.5 ± 3.2	3.4 ± 0.4	10.2 ± 1.3	1510 ± 260
t-Test	$P < 0.2$	NS	$P < 0.01$	NS	NS	NS	NS	NS[b]
Neutral lipid								
P388	5.5 ± 2.5	24.9 ± 1.5	3.9 ± 0.4	21.3 ± 2.3	36.5 ± 3.6	2.0 ± 1.2	5.8 ± 3.8	203 ± 27
P388/ADR	7.4 ± 3.8	24.1 ± 2.2	5.2 ± 1.1	18.4 ± 2.9	37.6 ± 5.0	1.8 ± 0.3	5.3 ± 1.4	135 ± 21
t-Test	NS	NS	$P < 0.02$	NS	NS	NS	NS	$P < 0.01$

Values given as mean ± SD.
[a]N = 6 for percent data; n = 5 for content data.
[b]Paired analysis showed a 17% difference in PL content, $P < 0.01$.

TABLE 2. Fatty acid distribution and lipid content of cells grown in 5% fetal calf serum

Lipid	Fatty Acid 14:0	16:0	16:1	18:0	18:1	18:2	20:4	Total Content
	% of total fatty acids							*μg/10⁸ cells*
Phospholipid								
P388[a]	1.7 ± 0.1	13.7 ± 0.4	3.7 ± 0.2	20.3 ± 0.4	56.4 ± 0.8	1.1 ± 0.1	3.2 ± 0.2	900 ± 160
P388/ADR	4.1 ± 0.4	15.4 ± 0.5	6.2 ± 0.4	17.9 ± 1.1	52.2 ± 1.1	1.2 ± 0.1	3.0 ± 0.2	1260 ± 260
t-Test	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS	$P < 0.02$
Difference[b]	141%	12%	68%	−12%	−7%	9%	−6%	
Neutral lipid								
P388	4.2 ± 0.5	26.5 ± 1.9	3.3 ± 0.3	21.5 ± 0.8	42.0 ± 2.0	0.3 ± 0.2	2.1 ± 0.7	82 ± 9
P388/ADR	8.1 ± 1.2	30.1 ± 1.9	5.6 ± 1.5	17.9 ± 1.8	36.2 ± 2.7	0.3 ± 0.1	1.8 ± 0.3	73 ± 9
t-Test	$P < 0.01$	$P < 0.02$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS	NS	NS
Difference[b]	93%	14%	70%	− 17%	− 14%	0	− 14%	

Values given as mean ± SD.
[a] N = 6
[b] $\frac{(\text{ADR} - \text{P388})}{\text{P388}} \times 100$

Acetate incorporation into fatty acids

Since the differences in fatty acid content did not arise from lipids in culture media or from differences in fatty acid incorporation, we evaluated fatty acid synthesis in sensitive and resistant cells. In order to examine de novo synthesis of fatty acids, P388 cells were transferred to fresh media and incubated with [1-^{14}C]acetate for 24 h (0–24 or 24–48). Lipids were extracted and hydrolyzed, and fatty acids were separated by HPLC as described in Materials and Methods. Three major peaks of radioactivity were found at retention times corresponding to coeluting myristic and palmitoleic, coeluting palmitic and oleic, and stearic acid. No radioactivity above background was found at the positions of docosahexaenoic, arachidonic, or linoleic acid. But several minor peaks were observed at longer retention times, one of which was identified as cholesterol. For P388/ADR, a greater proportion of ^{14}C was found in the coeluting myristic and palmitoleic acid peak than for P388 cells. The difference was greater for 0–24-h samples (14.6% ± 1.0 vs 9.7% ± 0.8, n = 7, $P < 0.01$) than for 24–48-h samples (10.1% ± 2.9 vs 7.4% ± 1.0, $P < 0.05$). At the earlier time, there was a lower proportion of ^{14}C found in stearic acid from P388/ADR than from P388 (22.1% ± 1.7 vs 27.1% ± 2.0, $P < 0.01$).

The distribution of ^{14}C between coeluting fatty acids was determined by hydrogenating the samples, separating the product by HPLC, and solving the resulting simultaneous linear equations (**Table 4**). A greater proportion of the ^{14}C was found in myristic acid for P388/ADR than for P388 cells. For 0–24-h samples, P388/ADR cells also had a greater proportion of ^{14}C in palmitoleic and less in stearic acid. Although the proportion of ^{14}C in oleic acid was similar for P388 and P388/ADR cells, the ratio of ^{14}C in oleic to that in stearic acid was greater for P388/ADR (1.5 ± 0.2) than for P388 cells (1.14 ± 0.2, $P < .05$) in 0–24 h-samples.

In the presence of 40 nM doxorubicin, P388 cell numbers were 13% and 28% lower after 24 and 48 h, respectively, than in the absence of doxorubicin. However, the distribution of ^{14}C between fatty acids was unaffected (Table 4). Similarly 2000 nM doxorubicin resulted in 9% and 20% lower P388/ADR cell numbers after 24 and 48 h, respectively, without affecting the distribution of ^{14}C.

While analyzing the initial acetate incorporation experiments, another interesting difference was found between

TABLE 3. Difference in rates of fatty acid uptake

	P388/ADR vs P388 (n = 7)	P388 ± Drug (n = 3)	P388/ADR ± Drug (n = 3)
		%	
Mean ± SD	−12 ± 16	4.1 ± 16.7	10.7 ± 7.7
$P < 0.05$ cutoff	15[a]	42	19
Power of test	20[a]	50	23

One μCi of 1-^{14}C-labeled fatty acid in FCS was added to cells as they were suspended in fresh media. An average rate of uptake was determined for 0–24 h and used in a paired analysis with simultaneously executed controls. P388/ADR cells were compared to P388 and cells with drug to those without. Forty nM doxorubicin was used for P388 cells and 2000 nM was used for P388/ADR cells.

[a] With this SD, it would have taken an actual difference of 20% to have an 80% chance of measuring a significant difference at the $P < 0.05$ level (i.e., ≥ 15%). Therefore, over the first 24 h there was less than 20% difference in the rates of uptake of these cell lines.

TABLE 4. Relative incorporation of [1-^{14}C]acetate into various fatty acids

	14:0	16:0	16:1	18:0	18:1
0-24 h					
P388 (n = 4)	6.0 ± 0.8[a]	33.2 ± 2.9	4.0 ± 0.8	26.5 ± 2.1	30.2 ± 4.3
P388 + drug (n = 3)	6.3 ± 0.6	36.7 ± 1.5	3.3 ± 1.2	28.3 ± 0.6	25.3 ± 0.6
P388/ADR (n = 4)	9.8 ± 0.5	31.8 ± 4.3	5.5 ± 0.7	21.2 ± 1.7	31.8 ± 3.7
P388/ADR + drug (n = 3)	10.3 ± 0.6	35.7 ± 1.5	4.7 ± 1.5	21.7 ± 1.2	27.7 ± 0.6
P388/ADR vs P388	$P < 0.005$		$P < 0.025$	$P < 0.005$	
24-48 h					
P388 (n = 3)	4.7 ± 1.2	27.3 ± 5.1	2.0 ± 1.7	29.7 ± 3.2	36.3 ± 7.5
P388 + drug (n = 3)	4.7 ± 0.6	24.0 ± 2.6	2.7 ± 1.2	28.3 ± 2.5	40.7 ± 5.0
P388/ADR (n = 3)	7.0 ± 1.0	25.7 ± 2.5	2.0 ± 1.0	29.7 ± 2.5	35.7 ± 1.2
P388/ADR + drug (n = 3)	5.7 ± 0.6	22.7 ± 2.3	3.7 ± 1.5	28.0 ± 1.0	40.0 ± 2.6
P388/ADR vs P388	$P < 0.05$				

Five μCi of [1-^{14}C]acetate was added to cells either 0 or 24 h after dilution with fresh media, and cells were harvested 24 h later. Doxorubicin concentrations were 40 nM for P388 and 2000 nM for P388/ADR. Statistical results are shown only for samples without doxorubicin.

[a]Mean ± SD of percentage incorporation.

these cell lines. A large fraction of ^{14}C was not recovered and presumably the acetate was oxidized to CO_2 in the mitochondrial tricarboxylic acid cycle. (When incubated for 24 h in media without cells, 100% of the ^{14}C was recovered.) Loss of ^{14}C was greater and incorporation into cellular components was lower for P388/ADR cells than for P388 (**Table 5**). This is consistent with greater metabolic activity for the resistant cells. These observations and interpretations were confirmed by examining rates at which these cell lines consumed O_2 (see below). Of the ^{14}C recovered in the cell pellet, the largest portion was in the lipid fraction (soluble in chloroform-methanol 2:1) with a smaller amount in nonlipid components (retained on a Whatman #1 filter). In one experiment, the lipid component was separated by TLC into phospholipids, triglycerides, and cholesterol. For both cell lines, the distribution of ^{14}C was 75% in phospholipid, 15% in triglyceride, and 6% in cholesterol for 0-24 h, and 85%, 5%, and 6% respectively, for 24-48 h.

TABLE 5. Differences in utilization of [1-^{14}C]acetate (paired analysis P388/ADR vs P388)

	Loss of ^{14}C[a]	Lipid Incorporation	Non-Lipid Incorporation
0-24 h (n = 9)			
Base rate[b]	31%	19%	13%
Paired analysis[c]	50 ± 34%	−24 ± 19%	−31 ± 22%
	$P < 0.01$	$P < 0.01$	$P < 0.01$
24-48 h (n = 8)			
Base rate[b]	22%	26%	13%
Paired analysis[c]	65 ± 40%	−20 ± 22%	−9 ± 22%
	$P < 0.01$	$P < 0.05$	

Incorporation of acetate was conducted as in Table 4 and a paired analysis was performed on the utilization of [1-^{14}C]acetate. About 42% (33% for the second day) of the ^{14}C was recovered in the media supernatant. Cell numbers (growth) of P388/ADR averaged only 5% less than those of P388 after 48 h (SD = 16).

[a]Presumably to CO_2 in the TCA cycle.

[b]Mean % of added ^{14}C that was utilized per 10^8 P388 cells.

[c]Mean ± SD of % difference, (P388/ADR-P388)/P388.

The effect of doxorubicin on the utilization of [1-^{14}C]acetate was examined. The concentrations used, 40 nM for P388 and 2000 nM for P388/ADR, resulted in 10% lower cell concentrations after 24 h for both cell lines. After 48 h, cell growth was inhibited by 20-25% for P388 and by 10-50% for P388/ADR. For 0-24-h samples, the only significant effect of doxorubicin was a small increase in the loss of ^{14}C for P388 cells (**Table 6**). For 24-48-h samples, doxorubicin increased rates of utilization of [1-^{14}C]acetate for oxidation (presumably to CO_2) and for incorporation into both lipid and nonlipid cellular components for both cell lines. The significance of this result, other than reflecting higher metabolic rate in resistance cells, is not clear.

Survey of other cell lines

Our experience with P388 and P388/ADR cells indicated that radioactive acetate incorporation experiments yielded results consistent with fatty acid distribution and content obtained by gas-liquid chromatography (see above). That is, for P388/ADR, a greater proportion of ^{14}C was found in myristic and palmitoleic acids, while with gas-liquid chromatography, the fatty acid distribution showed larger proportions of these same fatty acids in phospholipid (Tables 1 and 4). Because we found that [1-^{14}C]acetate incorporation was easier to perform, we surveyed five other pairs of drug-resistant/sensitive cell lines for changes in fatty acid pools. The incubation period in each case was 24 h after the addition of fresh media (see Methods). P388/AZQ^{30}U, N417/VP-16, and DC3F/AD X cells incorporated proportionally more ^{14}C into myristic acid than did their drug-sensitive, parent cell lines (**Table 7**). On the other hand, the ^{14}C distribution in CH^{R}C5 and HL60/AR cells was similar to that in their parent lines. Because the cell line DC3F/AD X is grown in doxorubicin, two experiments were performed with cells grown without drug. The results were not different from cells grown in doxorubicin (Table 7).

TABLE 6. Effect of doxorubicin on fate of [1-^{14}C]acetate in cell culture

	Loss of ^{14}C[a]	Lipid Incorporation	Non-Lipid Incorporation
P388			
0-24 h (n = 5)	13 ± 6%[b] $P < 0.01$	7 ± 12%	8 ± 9%
24-48 h (n = 4)	23 ± 4% $P < 0.01$	17 ± 1.4% $P < 0.01$	23 ± 19% $P < 0.05$
P388/ADR			
0-24 h (n = 6)	0.2 ± 2%	−3 ± 18%	0.2 ± 14%
24-48 h (n = 7)	44 ± 38% $P < 0.05$	44 ± 25% $P < 0.01$	22 ± 28%

Incorporation of acetate was conducted as in Table 4 and a paired analysis was performed on the utilization of [1-^{14}C]acetate. For P388/ADR 24-48-h samples, standard deviations were large as they were for the effect of doxorubicin on cell multiplication. Inhibition of P388/ADR cell growth after 48 h correlated with [1-^{14}C]acetate utilization (correlation coefficient to 0.85 ($P < 0.02$) for 1-^{14}C loss to Co_2 and 0.95 ($P < 0.001$) for lipid incorporation).

[a]Presumably to CO_2 in the TCA cycle.

[b]Mean ± SD of (with doxorubicin − control)/control.

Cells derived from the P388 line were the only ones to lose a noticeable amount of acetate, presumably to CO_2, indicating greater metabolic activity. The other five cell lines incorporated less acetate into fatty acids or into other cellular components. HL60 cells incorporated very little acetate when grown in serum-supplemented media.

Myristoyltransferase activity

Three separate preparations of P388 and P388/ADR cells were assayed for activity of the enzyme that transfers myristic acid from acyl-CoA to the N-terminus of certain proteins. The reason for this assay was to search for a reason for elevated pools of myristic acid found in P388/ADR cells. The hypothesis was that the large pools could be used to myristoylate proteins which, in turn, can have a function in drug detoxification. We found that P388 cells had a greater activity (31 ± 2 mU/mg protein) than P388/ADR (26 ± 2 mU/mg, $P < 0.05$). A paired analysis showed this difference, −15.6% ± 0.5, to be highly significant ($P < 0.001$). The cell extracts had similar protein contents (2.3 ± 0.2 vs 2.4 ± 0.5 mg/10^8 cells).

Acyl-CoA 9-desaturase activity

The differences found in the synthesis of palmitoleic and oleic acids in [1-^{14}C]acetate incorporation experiments were investigated by assaying for desaturase activity. Positive results would indicate that, in P388/ADR cells, there exists a mechanism to increase desaturation. To compare acyl-CoA 9-desaturase activities in P388 and P388/ADR cells, they were incubated with [1-^{14}C]palmitic acid for 4 h. The distribution of ^{14}C in various fatty acids was determined and the ratios 16:1/16:0 and 18:1/18:0 were used as indicators of desaturase activity. For P388 cells, both indicators were lower after transfer to fresh media (1-5 h) but rebounded by 24-28 h to levels greater than before transfer (**Fig. 1**). P388/ADR cells showed a similar pattern of activity but had greater desaturase activity at 24-28 h than did P388. Thus, resistant cells tend to increase unsaturated fatty acids at least in 16- and 18-carbon chains.

Acyl-CoA 9-desaturase activity experiments were also performed in the additional 5 lines surveyed for acetate incorporation. In some cases, there were indications of alterations in acyl-CoA 9-desaturase activity. But these altera-

TABLE 7. Relative incorporation of [1-^{14}C]acetate into various fatty acids

Cells	n	14:0	16:0	16:1	18:0	18:1
P388	4	5.9 ± 0.8[a]	31.8 ± 2.3	4.0 ± 0.4	24.0 ± 2.1	34.6 ± 4.3
P388/AZQ^{30}U	3	7.7 ± 1.0 $P < 0.05$	31.7 ± 4.1	3.9 ± 1.2	24.8 ± 0.5	31.9 ± 4.3
N417	3	6.0 ± 0.4	37.2 ± 2.9	13.6 ± 3.0	13.3 ± 0.3	30.0 ± 0.2
N417/VP-16	3	9.3 ± 1.0 $P < 0.005$	44.2 ± 1.7 $P < 0.05$	12.5 ± 1.0	11.0 ± 1.3 $P < 0.05$	23.0 ± 2.5 $P < 0.01$
DC3F	2	2.4 ± 0.1	38.2 ± 0.5	7.3 ± 2.1	28.2 ± 2.6	23.9 ± 0.1
DC3F/AD X	2	13.1 ± 3.5 $P < 0.025$	49.1 ± 5.2	5.0 ± 0.6	20.9 ± 3.0	12.0 ± 5.2
DC3F/AD X (no drug)	2	13.0 ± 1.3 $P < 0.005$	48.7 ± 0.3 $P < 0.01$	0.8 ± 0.9	26.8 ± 2.1	10.8 ± 2.8 $P < 0.05$
AUX B1	2	6.8 ± 1.2	40.0 ± 5.4	2.4 ± 1.3	30.7 ± 0.4	20.0 ± 4.8
CH^{R}C5	2	5.8 ± 2.6	39.6 ± 4.2	4.3 ± 0.1	28.3 ± 1.4	22.0 ± 5.5
HL60	1	7.2	30.5	30.1	7.9	24.3
HL60/AR	1	6.8	28.9	29.6	12.4	22.3
10% FCS						
HL60	1	6.9				
HL60/AR	3	6.1 ± 0.8				

[1-^{14}C]Acetate was added to flasks of drug-resistant and -sensitive cells immediately after addition of fresh media. Cells were harvested 24 h later and incorporation of ^{14}C into various fatty acids was analyzed. DC3F/AD X cells were tested both with the standard 10 μg/ml actinomycin D in the media and 0 or 3 days after removal of drug from the media. HL60 and HL60/AR cells were tested both in serum-free media and in media containing 10% FCS.

[a]Mean ± SD of percentage.

tions were not consistent. That is, there was greater activity in CH^{R}C5 cells where no ^{14}C incorporation changes were observed, and lower activity in DC3F/AD X cells where ^{14}C incorporation was higher than parental line CD3F.

O_2 consumption

The loss of ^{14}C from [1-^{14}C]acetate in drug-resistant (P388/ADR) cells was greater than for sensitive (P388) cells presumably because of greater rates of oxidation to Co_2. To investigate this assumption, the rates of O_2 consumption of these two cell lines were studied. Rates were measured over a 10-min period and were only slightly affected by changes in media O_2 content in the range used. P388/ADR cells consumed O_2 at twice the rate of P388 cells (3.0 ± 0.4 vs 1.4 ± 0.4, O_2 consumed/min per 3×10^7 cells with 100% equal to the O_2 content of air-saturated media, n = 4, $P < 0.01$). In one experiment, the use of media in which the cells had been growing for 4 days was compared with the use of fresh media. No effect was seen on rates of O_2 consumption. In another experiment, cells in early log phase growth were used (i.e., 24 h afters suspension in an equal volume of fresh media). Rates of O_2 consumption were twice that of cells before suspension in fresh media, but the rate for P388/ADR was still greater than that for P388 (5.3 vs 3.5). This result coupled with the greater loss of ^{14}C from acetate in P388/ADR cells, indicates that resistant cells have a higher oxidative metabolism.

DISCUSSION

Doxorubicin-resistant P388/ADR cells differed from P388 cells in that the former possessed greater relative amounts of myristic and palmitoleic acid. Although on a proportional basis these differences are large, these fatty acids are relatively minor ones and were overlooked by others (12).

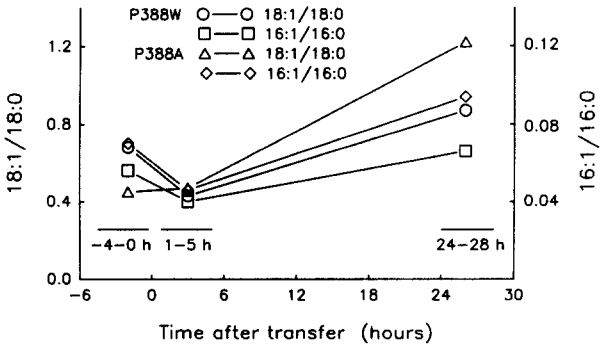

Fig. 1. Acyl-CoA 9-desaturase activity in cultures of P388 and P388/ADR (labeled A) cells measured using [1-^{14}C]palmitic acid as substrate and a 4-h incubation at various times after transfer to fresh media. Ratios of 1-^{14}C in 16:1/16:0 and in 18:1/18:0 were used as indicators of desaturase activity. Each point represents an average of three to five experiments. For P388 cells, standard deviations are 0.07–0.10 for the ratio 18:1/18:0 and 0.006–0.008 for the ratio 16:1/16:0. Standard deviations for P388/ADR cells are twice as great (0.045) for the 4-0-h 16:1/16:0 ratio. For 24–48 h, both ratios are greater for P388/ADR than for P388, $P < 0.025$ each.

That this result is not caused by differences in the rate of uptake of fatty acids from the media was shown here by using two experimental approaches: changing the proportion of serum in the media and incorporation of radiolabeled fatty acids.

The supply of fatty acids from the medium was reduced and fatty acid metabolism was altered for cells grown in 5% FCS rather than 15% FCS. However, the difference in relative amounts of myristic and palmitoleic acid between P388 and P388/ADR cells was as great or greater for cells grown in 5% FCS. When measured directly, the relative rates at which P388 and P388/ADR cells take up free fatty acid from the media were similar. In addition, no noticeable catabolism of fatty acids occurred. These results suggest that the differences in relative amounts of myristic and palmitoleic acid are not due to differences in uptake of fatty acids from the media, but rather to differences in de novo synthesis of fatty acids. This change may arise from a more basic genetic change that may ultimately influence membrane fluidity and thus, presumably, passive drug transport.

The fatty acid synthetase system can often produce myristic acid, although palmitic and stearic are more prominent products (28–30). When de novo synthesis of fatty acids was examined using the labeled precursor, [1-^{14}C]acetate, P388/ADR cells incorporated proportionally more ^{14}C into myristic acid than did P388 cells. Three of the five other drug-resistant cell lines tested also incorporated proportionally more acetate into myristic acid than did their sensitive, parent cell lines. This alteration is independent of the P180 glycoprotein involved with the multidrug resistance, efflux pump. That is, the alteration is present in DC3F/AD X but not in CH^{R}C5 cells, both of which have well-characterized increases in the P180 glycoprotein. In addition, the myristic acid alteration is present in P388/AZQ^{30}U cells, which do not exhibit an increase in the P180 glycoprotein (17). There is an example in the literature where the fatty acid synthetase system is modulated to produce more myristic acid when it is needed (31). Myristic acid is used by bioluminescent bacteria in the production of light. Induction of a specific acyltransferase is used to increase myristic acid production without altering production of other fatty acids.

One possible link between differences in myristic acid production and resistance to drugs is the cotranslational addition of myristic acid to certain proteins (32). Proteins in this group include cAMP-dependent protein kinase, cytochrome b_5 reductase, which is part of the acyl-CoA desaturase system, and certain guanine nucleotide-binding proteins. At present, the only known role of the myristic acid group in these proteins is to aid in their association with membranes. However, the high specificity for myristic acid, its presence in soluble enzymes, and its presence in proteins involved in signal transduction argue for other roles. A recent report implicates protein myristoylation in the priming of macrophages for secretion of arachidonic acid metabolites (33). As seen above, the cellular content

and synthesis of myristic acid is greater in P388/ADR than in P388 cells but the activity of the enzyme that attaches myristic acid to proteins is lower in the resistant cells. One possibility is that the higher pools of myristic acid partially inhibit myristoyl transferase thus decreasing myristoylated proteins. Future work should focus on detecting these proteins and delineating their function. The fatty acid extraction procedures used in this study would not extract proteins with covalently bound myristic acid.

Acyl-CoA 9-desaturase activity, and therefore production of palmitoleic acid, increased during the time period from 1 to 24 h after transfer of cells to fresh media. At this time, cells are shifting from a largely quiescent to an exponentially growing state. It could be that P388/ADR has a shorter lag time before the increase in desaturase activity, but that the activity for P388 cells reaches the same maximal levels early in the second day. This would account for the difference in desaturase activity when measured over 0–24 h ([1-^{14}C]acetate experiment) and 24–28 h ([1-^{14}C]palmitate experiment) but equal activities over 1–5 and 24–48 h. The transitory nature of this difference accounts for the failure to observe differences in the relative amount of oleic acid in these cell lines. The cellular pool of palmitoleic acid is much smaller than that of oleic and is therefore more easily perturbed. Due to the role in the desaturase system of myristoylated cytochrome b_5 reductase, myristic acid may be involved in the difference found in desaturase activity.

The data presented here show that P388/ADR cells have more phospholipid and less triglyceride than P388 cells. The greater phospholipid content could be due to the larger size of P388/ADR cells (at least 13% larger volume, data not shown). The lower triglyceride content of P388/ADR cells could be due to its use for phospholipid synthesis. On the other hand, the lower triglyceride content could also be related to their higher metabolic rate. These results are in contrast to those reported by Ramu, Glaubiger, and Weintraub (11), who found 3.6 times as much triglyceride in P388/ADR cells as in P388. The reason for this discrepancy is unknown, although there are some differences in our methodologies. The availability of fatty acids in the media affects the triglyceride content of these cells and could be a factor. Other workers failed to find major differences between P388/ADR and P388 cells (12). Their study would not have detected differences in triglyceride content of the magnitude found in the present study, but should have detected differences as large as those found by Ramu et al. (11).

Doxorubicin-resistant P388/ADR cells consume more O_2 and lose more radioactive acetate presumably to CO_2 than sensitive P388 cells. Acetate would be converted to acetyl-CoA and oxidized to CO_2 in the mitochondrial tricarboxylic acid cycle. The resulting NADH is used along with O_2 in the production of ATP. The other cell lines studied in this report did not lose as much radioactive acetate. Presumably they use glycolysis as their major source of energy. Further comparisons with these cell lines could examine rates of energy usage (e.g., rates of turnover of ATP). It is reasonable to expect that resistant cells require more energy to power their resistance mechanisms; for example, a constantly active efflux pump or elevated rates of repair. Vinblastine-resistant human leukemic T lymphoblasts have been shown to consume more O_2 than sensitive cells as do their isolated mitochondria (34). Also, greater rates of glycolysis were found in doxorubicin-resistant MCF-7 human breast cancer cells than in the sensitive cell line (35). Thus the greater energy requirements of drug-resistant cancer cells may be a common phenomenon.

Differences in rates of energy production must be kept in mind while analyzing other metabolic observations. In the present case, P388/ADR cells incorporated less ^{14}C from acetate into cellular lipids than did P388 cells. However, rates of fatty acid or cholesterol synthesis were not necessarily lower. The specific activity of the [1-^{14}C]acetate could have been diluted by acetyl-CoA, the flux of which (production from pyruvate and utilization in the tricarboxylic acid cycle) is apparently greater in P388/ADR cells. Similarly, the effect of doxorubicin on these cells could be either a general increase in the utilization of acetate for all purposes or a decrease in the dilution of specific activity by acetyl-CoA produced from other compounds. The latter could be accomplished by inhibition of the pyruvate dehydrogenase system.

The major point of this report is that greater proportions of myristic acid are found in the products of fatty acid synthesis in doxorubicin-resistant cells as compared to parent cells. This is also true of some other resistant lines, but not a general phenomenon. The significance of this finding lies in membrane alterations that can result in altered transport. Because these fatty acid alterations do not correlate with the presence of P-glycoprotein overexpression, it implies that altered myristic acid pools may be involved in yet another unknown mechanism of resistance that involves membranes. On the other hand, the difference in myristic acid pools may be a byproduct of a more important biochemical reaction and the increase in myristic acid is not crucial. Further research should examine the cellular content of myristoylated proteins in drug-resistant and -sensitive cell lines. Either the overall level of myristoylation or more likely the presence of specific myristoylated proteins could be the next link in the mechanism of drug resistance. A secondary point of this report is that P388/ADR cells have greater rates of oxidative metabolism than P388 cells. The significance of this finding is that, since the use and especially the synthesis of myristic acid requires energy, it is possible that there is a connection between metabolic rate and the fatty acid synthetase system. It also points to a requirement for energy to fuel mechanism(s) of resistance.

Supported by the American Cancer Society, Grant CH-366, and by the National Cancer Institute, DHHS, PHS grant number CA33681. We thank Dr. Martha G. Price, Sunita Hanjura, and Michele Pethel who performed extensive preliminary studies leading us to this

area of investigation. We also thank Drs. June Beidler, Austin Doyle, Victor Ling, and Alex Hindenberg for permission to use the cell lines developed in their laboratories. We thank Dr. Ronald Felsted and Constance Glover for analysis of samples for myristoyltransferase activity.

Manuscript received 20 July 1989 and in revised form 23 April 1990.

REFERENCES

1. Weisenthal, L. M., P. L. Dill, J. Z. Finklestein, T. E. Duarte, J. A. Baker, and E. M. Moran. 1986. Laboratory detection of primary and acquired drug resistance in human lymphatic neoplasms. *Cancer Treat. Rep.* **70:** 1283-1295.
2. Kessel, D., and T. Corbett. 1985. Correlations between anthracycline resistance, drug accumulation and membrane glycoprotein patterns in solid tumors of mice. *Cancer Lett.* **28:** 187-193.
3. Beck, W. T. C. 1983. Vinca alkaloid-resistant phenotype in cultured human leukemic lymphoblasts. *Cancer Treat. Rep.* **67:** 875-882.
4. Skovsgaard, T. 1978. Mechanism of cross-resistance between vincristine and daunorubicin in Ehrlich ascites tumor cells. *Cancer Res.* **38:** 4722-4747.
5. Kartner, N., J. R. Riordan, and V. Ling. 1983. Cell surface *p*-glycoprotein associated with multidrug resistance in mammalian cell lines. *Science.* **221:** 1285-1288.
6. Ling, V. 1982. Genetic Basis of Drug Resistance in Neoplasia. CRC Press, Boca Raton, FL. p. 1.
7. Riordan, J. R., and V. Ling. 1985. Genetic and biochemical characteristics of multidrug resistance. *Pharmacol. Ther.* **28:** 51-75.
8. Curt, G. A., N. J. Clendeninn, and B. A. Chabner. 1984. Drug resistance in cancer. *Cancer Treat. Rep.* **68:** 87-99.
9. Thompson, M. G., S. B. Chahwala, and J. A. Hickman. 1987. Inhibition of human erythrocyte inositol lipid metabolism by Adriamycin. *Cancer Res.* **47:** 2799-2803.
10. Zhao, F. K., L. F. Chuang, and R. Y. Chuang. 1988. Mechanism of inhibition of protein kinase C activity by Adriamycin. *Proc. Am. Assoc. Cancer Res.* **29:** 271.
11. Ramu, A., D. Glaubiger, and H. Weintraub. 1984. Differences in lipid composition of doxorubicin-sensitive and -resistant P388 cells. *Cancer Treat Rep.* **68:** 637-641.
12. Holleran, W. H., M. W. DeGregorio, R. Ganapathi, J. R. Wilbur, and B. A. Macher. 1986. Characterization of cellular lipids in doxorubicin-sensitive and -resistant P388 mouse leukemia cells. *Cancer Chemother. Pharmacol.* **17:** 11-15.
13. Tapiero, H., Z. Mishal, M. Wioland, A. Silber, A. Fourcade, and G. Zwingelstein. 1986. Changes in biophysical parameters and in phospholipid composition associated with resistance to doxorubicin. *Anticancer Res.* **6:** 649-652.
14. Wright, L., M. Dyne, K. Holmes, and C. Mountford. 1985. Phospholipid and ether-linked phospholipid content alter with cellular resistance to vinblastine. *Biochem. Biophys. Res. Commun.* **133:** 539-545.
15. Vrignaud, P., D. Montaudon, D. Londos-Gagliardi, and J. Robert. 1986. Fatty acid composition transport and metabolism in doxorubicin-sensitive and -resistant rat glioblastoma cells. *Cancer Res.* **46:** 3258-3261.
16. Johnson, R. K., M. P. Chitnis, W. M. Embrey, and E. B. Gregory. 1978. In vivo characteristics of resistance and cross-resistance of an Adriamycin-resistant subline of P388 leukemia. *Cancer Treat. Rep.* **62:** 1535-1547.
17. Gutierrez, P. L., P. J. Wilder, and N. Biswal. 1989. In vitro multidrug resistance of P388 murine leukemia selected for resistance to diaziquone. *Cancer Commun.* **3:** 181-190.
18. Carney, D. N., A. F. Gazdar, G. Bepler, J. G. Guccion, P. J. Marangos, T. W. Moody, M. H. Zweig, and J. D. Minna. 1985. Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res.* **45:** 2913-2923.
19. Biedler, J. L., and H. Riehm. 1970. Cellular resistance to actinomycin D in Chinese hamster cells in vitro: cross resistance, autoradiographic, and cytogenetic studies. *Cancer Res.* **30:** 1174-1184.
20. Ling V., and L. H. Thompson. 1974. Reduced permeability in CHO cells as a mechanism of resistance to colchicine. *J. Cell. Physiol.* **83:** 103-116.
21. Bhalla, K., A. Hindenberg, R. N. Taub, and S. Grant. 1985. Isolation and characterization of an anthracycline-resistant human leukemic cell line. *Cancer Res.* **45:** 3657-3662.
22. Breitman, T. R., S. J. Collins, and B. R. Keene. 1980. Replacement of serum by insulin and transferrin supports growth and differentiation of the human promyelocytic cell line, HL-60. *Exp. Cell. Res.* **126:** 494-498.
23. Soloni, F. G. 1971. Simplified manual micromethod for determination of serum triglycerides. *Clin. Chem.* **17:** 529-534.
24. Ames, B. N. 1966. Assay of inorganic phosphate, total phosphate and phosphatases. *Methods Enzymol.* **8:** 115-118.
25. Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J. Lipid Res.* **5:** 600-608.
26. Glover, C. J., C. Goddard, and R. L. Felsted. 1988. N-Myristoylation of $p60^{src}$: identification of a myristoyl-CoA:glycylpeptide N-myristoyltransferase in rat tissues. *Biochem. J.* **250:** 485-491.
27. Zevallos, M. G. and T. Farkas. 1989. Manipulation of plasma membrane physical state affects desaturase activity in rat lymphocytes. *Arch. Biochem. Biophys.* **271:** 546-552.
28. Barnes, E. M., and S. J. Wakil. 1968. Studies on the mechanism of fatty acid synthesis. XIX. Preparation and general properties of palmityl thioesterase. *J. Biol. Chem.* **243:** 2955-2962.
29. Bedord, C. J., P. E. Kolattukudy, and L. Rogers. 1978. Isolation and characterization of a tryptic fragment containing the thioesterase segment of fatty acid synthetase from the uropygial gland of goose. *Arch. Biochem. Biophys.* **186:** 139-151.
30. Smith, S., and S. Abraham. 1971. Fatty acid synthetase from lactating rat mammary gland. II. Studies on the termination sequence. *J. Biol. Chem.* **246:** 2537-2542.
31. Byers, D. M. 1988. Luminescence-specific sythesis of myristic acid in the bioluminescent bacterium *Vibrio harveyi*. *Biochem. Cell Biol.* **66:** 741-749.
32. Towler, D. A., J. I. Gordon, S. P. Adams, and L. Glaser. 1988. The biology and enzymology of eukaryotic protein acylation. *Annu. Rev. Biochem.* **57:** 69-99.
33. Aderem, A. A. 1988. Protein myristoylation as an intermediate step during signal transduction in macrophages: its role in arachidonic acid metabolism and in responses to interferon gamma. *J. Cell Sci.* **Supplement 9:** 151-167.
34. Wright, L. C., M. Dyne, K. T. Holmes, T. Romeo, and C. E. Mountford. 1986. Cellular resistance is associated with altered respiratory function. *Biochem. Int.* **13:** 295-305.
35. Lyon, R. C., P. F. Daly, P. J. Faustino, J. S. Cohen, and C. E. Meyers. 1987. Metabolic changes associated with drug resistance in human breast cancer cells monitored by magnetic resonance spectroscopy. *Proc. Am. Assoc. Cancer Res.* **28:** 293.