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Enhanced Glycosylation Induced by Adriamycin

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SUMMARY

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Within 30 min of exposure of P388 murine leukemia cells to the anthracycline anti-tumor drug adriamycin, cell-surface electronegativity and incorporation of radioactive fucose into membrane glycoproteins were markedly increased, while cell surface hydrophobicity and incorporation of thymidine into DNA decreased. Incorporation of labeled leucine into cell protein was unaffected. These data indicate a drug-induced increase in production of electronegative and hydrophilic membrane glycoproteins which was not found when adriamycin-resistant cells were similarly treated.

INTRODUCTION

Adriamycin is a broad-spectrum anti-tumor antibiotic which is useful in the treatment of neoplastic disease in man (1). The major mode of drug action involves intercalation into the DNA helix, leading to inhibition of nucleic acid biosynthesis (2–4). Conversion of adriamycin to a free-radical form may be required both for its interaction with DNA (5) and for the drug-induced peroxidation of lipids which has been described (6–8). Treatment with adriamycin enhances the rate of Concanavalin A-induced agglutination of Sarcoma 180 cells (9). Properties of model membranes (10, 11) and erythrocyte membranes (11, 12) were also altered by the drug. Data reported therefore suggest a mode of action of adriamycin involving cell-surface and membrane phenomena (9–12).

Methods used in this laboratory for detection and characterization of cell-surface and membrane alterations caused by anti-tumor agents have previously been described (13–15). In the present study, effects of exposure to adriamycin of the drug-sensitive P388 murine leukemia and 2 drug-resistant sublines are examined.

MATERIALS AND METHODS

Cell lines. The P388, P388/ADR[1] and P388/VCR cell lines were obtained from Dr. I. Wodinsky, Arthur D. Little Corp., Cambridge, Mass., and were maintained by serial weekly transplant of 10^6 cells in $C_3D_2F_1$ male mice. Survival of animals bearing the P388 murine leukemia was significantly increased (to 235–260% of control) by treatment with 0.65 mg/kg of adriamycin via intraperitoneal injection. In contrast, lives of animals bearing P388/

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[1] The abbreviations used are: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate; ADR, adriamycin; VCR, vincristine; PEG, polyethylene glycol (mol. wt. 6000); PEG-palmitate, palmitate ester of PEG with 70% of available OH groups esterified; Con A, Concanavalin A.

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ADR (selected for adriamycin resistance) and P388/VCR (selected for vincristine resistance) were not prolonged by treatment with adriamycin (16, 17). In work reported in detail elsewhere (18), Inaba and Johnson found an LD_{99} (adriamycin concentration lethal to 99% of cells after 1 hour at 37° *in vitro*) of 0.25 μg/ml for P388 as compared with 200 μg/ml for the drug-resistant lines.

Chemicals. [^{14}C]-labeled thymidine and leucine were provided by New England Nuclear Corp. Stock solutions of 50 μM were prepared by dilution with carrier to obtain a specific activity of 10 μCi/ml. [^{3}H]-labeled L-fucose was purchased from New England Nuclear Corp.; stock solution (carrier-free) contained 100 μCi/ml. Adriamycin was supplied by the Division of Cancer Treatment, National Institutes of Health; 10 mg/ml stock solutions were stored under nitrogen at −20°. Dextran T 500 (lot 7863) was obtained from Pharmacia, polyethylene glycol (mol. wt. 6000) from Pierce Chemical Co., electrophoresis supplies from Ortec, and marker proteins from Sigma Chemical Co.

Incubations. Suspensions of freshly-isolated cells in HEPES-buffered Fisher's medium (pH 7.2) containing 10% horse serum were filtered through glass wool to remove clumps, and brought to a density of 2×10^6 cells/ml. One ml portions of this suspension were incubated at 37° with 0–30 μg/ml levels of adriamycin for 30 min. The cells were then collected by centrifugation, washed once with fresh medium, and used for further studies.

Adriamycin accumulation was measured by extraction of pellets of 2×10^6 cells with 500 μl of CHCl-EtOH (1:1). The debris was removed by centrifugation and the drug concentration in the supernatant fluid was determined fluorometrically (19).

Effects of prior exposure to adriamycin on incorporation of labeled precursors into acid-insoluble material were measured by suspending drug-treated cell pellets in fresh medium at 37°. To 1 ml suspensions of 2×10^6 cells were added the 10 μl stock solutions of substrates described above. After 5 min incubations with labeled thymidine and 30 min incubations with labeled fucose or leucine, the cells were collected by centrifugation. Thymidine and leucine incorporation was determined by washing pellets twice with 0.3 M $HClO_4$. The pellets were then solubilized (NCS, Amersham/Searle) for determination of radioactivity of liquid scintillation counting. Fucose incorporation into glycoproteins was measured (20) by three washings of cell pellets with 5% trichloroacetic acid, one wash with $CHCl_3$: methanol:ether (2:2:1) and one wash with methanol. The residue was solubilized and radioactivity determined as described above.

Membrane studies. After fucose labeling, cell membranes were isolated (21) and analyzed by polyacrylamide gel electrophoresis using 5% gels. After electrophoresis (2 mA/gel, 2 hours) the cylindrical gels (5 × 75 mm) were sliced into 1 mm sections and radioactivity in each section was measured by liquid scintillation counting. Protein standards (ovalbumin, bovine serum albumin, chymotrypsinogen, ferritin, and catalase) were run on similar gels which were then stained with Brilliant Coomassie Blue so that the relationship between molecular weight and migration could be estimated.

Alternatively, fucose-labeled cells were lysed, nuclei removed, and the post-nuclear fraction extracted with lithium diiodosalicylate to selectively solubilize glycoproteins as previously described (22). The glycoprotein fraction was purified by subsequent dialysis and extractions (22) and the level of radioactivity in this preparation was measured by liquid scintillation counting.

To determine the nature of the radioactive material in these membrane fractions, the purified membranes or diiodosalicylate extract was hydrolyzed in 0.5 ml of 1 N HCl at 100° for 60 min. The debris was removed by centrifugation, and the supernatant fluid was concentrated *in vacuo* and stored over NaOH pellets to remove traces of HCl. The radioactive material was then analyzed by paper chromatography as previously described (23).

Electrophoretic mobility. The electrophoretic mobility at the shear plane was monitored with the Mark II apparatus supplied by Rank Bros., Cambridge, U.K. Cells were suspended in a solution containing 4.5% sorbitol, 14.5 mM NaCl and 1 mM $NaHCO_3$ at pH 7.0 (24). The electropho-

retic mobility of individual cells was measured in both directions with change of polarity between measurements. At least 30 such measurements were made for each determination.

Partitioning studies. After treatment of cells with adriamycin as described above, pellets containing 10^6 cells were suspended in 100 μl of 150 mM NaCl and then added to a 9.9 g mixture of 5.0% (w/w) Dextran T-500 + 3.6% PEG made up in 140 mM NaCl + 10 mM sodium phosphate buffer at pH 7.0 and containing 0.001% PEG palmitate (27). After gentle mixing, a 1 ml portion was removed and the cell concentration measured with a Coulter Electronic Particle Counter, after appropriate dilution. The phases were then allowed to separate for 20 min at 22°. An aliquot of the upper phase was removed and the cell number measured as before. The partition coefficient is defined as the number of cells in the top phase expressed as a percent of the total cell number (25).

Transport studies. The effect of prior treatment with adriamycin on amino acid and nucleoside transport was determined as described previously (13).

Studies with another inhibitor. To ascertain whether effects seen in this study were produced by non-specific inhibition of DNA synthesis, freshly-isolated P388 cells and P388 sublines were incubated in medium containing 1 μM cytosine arabinoside for 30 min at 37°. These cells were then used for determination of electrophoretic mobility, partition coefficient, and for the measurement of incorporation of labeled thymidine, leucine and fucose into acid-insoluble macromolecules as described above.

RESULTS

All results are shown in terms of cell number. One gram of cells (wet weight) contained 2.4×10^8 P388, 3.0×10^8 P388/ADR or 3.1×10^8 P388/VCR cells. The Coulter Channelyzer 1000 indicated mean diameters of 11.25 μ for P388, 10.74 μ for P388/ADR and 10.48 μ for P388/VCR. Mr. I. Wodinsky, Arthur D. Little Corp., reported that typical preparations of these cells freshly isolated from mice contained less than 1% macrophages.

Adriamycin accumulation. Incubation for 30 min at 37° in medium containing 10 μg/ml of drug resulted in accumulation of 33 ± 2.4 μg of adriamycin per 10^7 P388 cells. For P388/ADR and P388/VCR, the corresponding numbers were 17 ± 1.4 and 18 ± 1.5 μg/10^7 cells.

Drug effects on precursor incorporation. Incorporation of labeled thymidine into DNA was inhibited by approximately 50% when P388 cells were treated first with 3 μg/ml of adriamycin for 30 min (Table 1). To produce the same effect in P388/ADR and P388/VCR, a drug level of 10 μg/ml was required. A 30 min incubation with drug levels as high as 30 μg/ml did not affect leucine incorporation into protein in any cell line.

Incorporation of fucose into glycoprotein was markedly stimulated by 0.3 μg/ml of adriamycin in the P388 cell line. The incorporation of fucose by untreated P388/ADR and P388/VCR cells was inherently greater than P388, and was not affected by adriamycin levels of 3–30 μg/ml.

When cells were incubated with radioactive fucose for 60 min, and membranes then isolated as described by Bosmann *et al.* (21), we found a recovery of total acid-insoluble radioactivity ranging from 70–78% in separate experiments involving all three cell lines. When the membrane material was subjected to analysis by gel electrophoresis (5% gels as described by Bosmann *et al.* [21]), all of the radioactivity incorporated during this brief labeling interval was found to migrate in a single peak. A comparison with the migration rate of several standard protein samples indicated an approximate molecular weight of 25,000.

Extraction of nuclei-free cell homogenates with lithium diiodosalicylate followed by removal of low molecular-weight materials (22) resulted in the recovery of 68% of radioactive fucosylated products in P388 cells, 74% in P388/ADR and 78% of P388/VCR. Paper chromatography of acid hydrolysates of isolated membranes or of the lithium salt extract indicated the radioactive product to be >90% fucose.

Electrophoretic mobility. The mobility of P388 cells was inherently less than that of P388/ADR and P388/VCR (Table 2).

TABLE 1

Effect of adriamycin on incorporation of radioactive precursors

Cells were incubated for 30 min with specified levels of adriamycin, then suspended in fresh medium and incubated for 5 min with [^{14}C]thymidine, or for 30 min with [^{14}C]-leucine or [^{3}H]fucose. Data are shown in terms of counts/min of radioactivity incorporated into acid-insoluble material per 10^7 cells.

Cell line	Drug level	Thymidine	Leucine	Fucose
	(μg/ml)			
P388[a]	0	6970[c]	1710	1450
	3	3000	1650	2670
	10	380	1510	2480
	30	350	1460	2220
P388/ADR[a]	0	11300	2050	6030
	3	10240	2030	6210
	10	5350	2080	6830
	30	1430	1960	7010
P388[b]	0	9950	2370	1900
	0.3	8905	2280	3805
	1.0	7510	2295	3790
	3	4250	2200	3750
	10	600	2100	3500
	30	400	2080	3050
P388/ADR[b]	0	10530	1940	5620
	0.3	9860	1910	5680
	1.0	9720	1930	5990
	3	9540	1900	6060
	10	5030	1890	6100
	30	1210	1860	6340
P399/VCR[b]	0	9920	2270	3570
	3	9620	2230	3690
	10	4900	2130	3710
	30	1820	2050	3970

[a] Experiment 1.

[b] Experiment 2.

[c] Data shown as average of three determinations (rounded to nearest 10).

Incubation with 0.3 μg/ml of adriamycin for 30 min substantially increased the electronegativity at the shear plane of the cell of the P388 cell, without affecting the other cell lines. After treatment with 10 μg/ml adriamycin, the electronegativity of P388 was actually slightly greater than that of the drug-resistant cells.

Partitioning behavior. To monitor changes in cell-surface properties not related to charge, a two-phase aqueous polymer system was employed. When the phases of this mixture separate, there is no potential difference across the interface and less than 2% of any cell line examined here will partition into the upper phase. Addition of 0.001% PEG-palmitate, which preferentially partitions into the PEG-rich upper phase (27–29) attracts cells with a sufficient number of hydrophobic palmitate binding sites at or near the cell surface. The data (Table 3) show P388/ADR and P388/VCR cells to be inherently less hydrophobic than P388. Treatment with 3 μg/ml adriamycin markedly reduced the hydrophobicity of P388 without affecting the other cell lines, a finding consistent with the hypothesis of a drug-induced increase in the level of certain hydrophilic glycoprotein on the P388 cell surface.

Transport effects. Transport of the nonmetabolized amino acid cycloleucine, and of the nucleoside uridine were not affected by levels of adriamycin as high as 30 μg/ml in any cell line examined here.

Effects of cytosine arabinoside. A 30 min incubation in medium containing 1 μM of this drug inhibited incorporation of a sub-

TABLE 2

Effect of adriamycin of electrophoretic mobility

Cells were incubated for 30 min with specified levels of adriamycin, then suspended in a sorbitol-based medium (described in the text) for determination of electrophoretic mobility by observation of migration rate of individual cells in an electric field. Each measurement shown involved 30 determinations with reversal of field.

Cell line	Adriamycin level	Electrophoretic mobility
	(μg/ml)	*(μm/V/cm/sec)*
P388	0	−1.65 ± 0.08[a]
	1.3	−1.88 ± 0.10
	1.0	−1.89 ± 0.15
	3	−1.90 ± 0.10
	10	−2.0 ± 0.09
P388/ADR	0	−1.95 ± 0.10
	3	−1.96 ± 0.07
	10	−1.98 ± 0.13
P388/VCR	0	−1.85 ± 0.07
	3	−1.85 ± 0.09
	10	−1.87 ± 0.11

[a] Data shown as mean ± S.D.

sequent 5 min pulse of [^{14}C]thymidine into DNA by 90–95% in all three cell lines without significantly affecting partitioning behavior, or electrophoretic mobility. The treatment with cytosine arabinoside did not significantly affect subsequent incorporation of labeled leucine or fucose into acid-insoluble pools of macromolecules.

DISCUSSION

The transplantable P388 murine leukemia was chosen for this study since it is highly responsive to adriamycin *in vivo*. For comparison, two drug-resistant sublines of P388 were also examined. Previous reports (9–12) indicated a site of action of adriamycin which appeared to involve components of the cell membrane. The present study has confirmed these reports; within 30 min of exposure of the P388 cell to adriamycin there is a marked change in two cell-surface properties, electronegativity and hydrophobicity. The incorporation of labeled fucose into cell glycoprotein was also enhanced.

These data indicate that exposure of P388 cells to adriamycin involves enhanced glycosylation of existing acceptors, but not an overall increase in net protein synthesis. The drug-resistant cells showed an inherently greater amount of fucosylation during the labeling period which was not affected by treatment with drug.

Cells made resistant (13, 14, 26–31) to any of a group of natural products which includes adriamycin, daunorubicin, chromomycin, actinomycin D, vincristine, emetine and vinblastine, are generally cross-resistant to the others, and show a greater rate of glycoprotein synthesis, an enhanced cell-surface electronegativity, less glycoprotein degradation and a higher level of glycosyltransferse activity than do parent cell lines (32–37). It has been suggested (34–36) that presence of an elevated level of membrane glycoprotein in the drug-resistant cells might alter membrane 'fluidity,' and thereby restrict drug uptake. But the major mode of drug resistance appears to involve an enhanced rate of *exodus* of accumulated drug (29–31, 37–39) rather than an uptake barrier. Whether this latter phenomenon is related to the findings reported here remains to be established.

We proposed that an important consequence of exposure of certain drug-responsive cells to adriamycin is an alteration in the normal pattern of glycoprotein synthesis, resulting in enhanced incorporation of at least one sugar into cell-membrane glycoprotein, increased cell-surface electronegativity, and decreased hydrophobicity. Selection for adriamycin resistance in two cases leads to an altered pattern of glycoprotein synthesis, an altered cell membrane, and 'resistance' to effects of the drug on these cell properties. The role of the drug-induced alterations in these and other membrane phenomena as factors in drug toxicity remains to be established.

TABLE 3

Partition coefficients of cell lines

Cells were incubated for 30 min with specified levels of adriamycin, then suspended in 0.9% NaCl and 0.1 ml aliquots containing 10^5 cells were mixed with 5 ml portions of each phase of the 2-phase system described in the text. Data represent percent of total cells which appeared in the upper phase after the phases had separated.

Cell line	Adriamycin level	Partition coefficient
	(μg/ml)	*(%)*
P388	0	25.5 ± 2.6[a]
	0.3	15.2 ± 2.1
	1.0	13.6 ± 1.8
	3	12.2 ± 1.9
	10	8.9 ± 0.9
	30	8.2 ± 0.6
P388/ADR	0	4.9 ± 0.4
	3	5.3 ± 0.5
	10	5.2 ± 0.4
	30	4.5 ± 0.3
P388/VCR	0	8.8 ± 0.6
	3	8.9 ± 0.5
	10	8.3 ± 0.5
	30	8.0 ± 0.6

[a] Results shown as average ± S.D. for five experiments.

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REFERENCES

1. Blum, R. H. (1975) An overview of studies with adriamycin (NSC 123127) in the United States. *Cancer Chemotherap. Repts.* **6** (part 3), 247–251.
2. Di Marco, A. (1975) Adriamycin (NSC 123127): mode and mechanism of action. *Cancer Chemother. Rep.* **6**, 91–106.
3. Meriwether, W. D. & Bachur, N. R. (1972) Inhibition of DNA and RNA metabolism by daunorubicin and adriamycin in the L1210 mouse leukemia. *Cancer Res.* **32**, 1137–1142.
4. Kim, S. H. & Kim, J. H. (1972) Lethal effect of adriamycin on the division cycle of HeLa cells. *Cancer Res.* **32**, 323–325.
5. Bachur, N. R., Gordon, S. L. & Gee, M. V. (1978) A general mechanism for microsomal activation of quinone anticancer agents to free radicals. *Cancer Res.* **38**, 1745–1750.
6. Goodman, J. & Hochstein, P. (1977) Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. *Biochem. Biophys. Res. Commun.* **77**, 797–803.
7. Myers, C. E., McGuire, W. & Young, R. (1976) Adriamycin: amelioration of toxicity by α-tocopherol. *Cancer Treatment Rep.* **60**, 961–962.
8. Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K. & Young, R. C. (1977) Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor responses. *Science* **197**, 165–167.
9. Murphree, S. A., Cunningham, L. S., Hwang, K. M. & Sartorelli, A. C. (1976) Effects of adriamycin on surface properties of sarcoma 180 ascites cells. *Biochem. Pharmacol.* **25**, 1227–1231.
10. Tritton, T. R., Murphree, S. A. & Sartorelli, A. C. (1977) Characterization of drug-membrane interactions using the liposome system. *Biochem. Pharmacol.* **26**, 2319–2323.
11. Goldman, R., Facchinetta, T., Bach, D., Raz, A. & Shinitzky, M. (1978) A differential interaction of daunomycin, adriamycin and their derivatives with human erythrocytes and phospholipid bilayers. *Biochim. Biophys. Acta* **512**, 254–269.
12. Schioppocassi, G. & Schwartz, H. (1977) Membrane actions of daunorubicin in mammalian erythrocytes. *Res. Commun. Chem. Pathol. Pharmacol.* **18**, 519–531.
13. Kessel, D. (1977) Effects of photo-activated porphyrins at the cell surface of leukemia L1210 cells. *Biochemistry* **15**, 3443–3449.
14. Kessel, D. (1977) Effects of acronycin on cell-surface properties of murine leukemia cells. *Biochem. Pharmacol.* **26**, 1077–1081.
15. Kessel, D. & McElhinney, R. S. (1978) Effect of dithiocarbanilates on some biological and biophysical properties of leukemia L1210 cell membranes. *Mol. Pharmacol.* **14**, 1121–1129.
16. Johnson, R. K., Chitnis, M. P. & Goldin, A. (1976) Characteristics of resistance and cross-resistance *in vivo* of a sub-line of P388 leukemia resistant to adriamycin. *Pharmacologist* **18**, 173.
17. Johnson, R. K., Ovejera, A. A. & Goldin, A. (1976) Activity of anthracyclines against an adriamycin INSC 123127)-resistant subline of P388 leukemia with special emphasis on Cinerubin A (NSC 18334). *Cancer Treatment Rep.* **60**, 99–102.
18. Inaba, M. & Johnson, R. K. (1978) Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline-resistant sublines of P388 leukemia. *Biochem. Pharmacol.* **27**, 2123–2130.
19. Bachur, N. R., Moore, A. L., Bernstein, J. G. & Liu, A. (1970) Tissue distribution and disposition of daunomycin in mice: fluorometric and isotopic methods. *Cancer Chemother. Rep.* **54**, 89–94.
20. Lazo, J. S., Hwang, K. M. & Sartorelli, A. C. (1977) Inhibition of L-fucose incorporation into glycoprotein of sarcoma 180 ascites cells by 6-thioguanine. *Cancer Res.* **37**, 4250–4244.
21. Chen, K., Tsai, C. & Canellakis, E. S. (1975) Comparison of physical and immunological properties of plasma membranes of two mouse leukemia cell lines, P388 and L1210. *Cancer Res.* **35**, 2403–2412.
22. Chen, K., Kramer, R. H. & Canellakis, E. S. (1978) Isolation and characterization of surface glycoproteins from L-1210, P-388 and HeLa cells. *Biochim. Biophys. Acta* **507**, 107–118.
23. Bosmann, H. B., Hagopian, A. & Eylar, E. H. (1968) Glycoprotein biosynthesis: the characterization of two glycoprotein fucosyl transferases in HeLa cells. *Arch. Biochem. Biophys.* **128**, 470–481.
24. Bosmann, H. B. (1972) Platelet adhesiveness and aggregation. II. Surface sialic acid, glycoprotein: n-acetylneuraminic acid transferase and neuraminidase of human blood platelets. *Biochim. Biophys. Acta* **279**, 456–474.
25. Walter, H. (1977) Partition of cells in two-polymer aqueous phases: a surface affinity method of cell separation, in *Methods of Cell Separation, Volume I.* Plenum Publ. Co., New York, 307–354.
26. Albertsson, P. A. (1971) *Partition of Cell Particles and Macromolecules.* Wiley-Interscience, New York.
27. Shanbhag, V. P. & Johansson, G. (1974) Specific extraction of human serum albumin by partition in aqueous biphasic systems containing poly(ethyleneglycol) bound ligand. *Biochem. Biophys. Res. Commun.* **61**, 1141–1146.
28. Walter, H. & Krob, E. J. (1977) Membrane surface

properties reflected by cell partition in two-polymer aqueous phases: classes of beef erythrocytes having different membrane lipid, charge and affinity for a ligand. *FEBS Lett.* **78**, 105–108.

29. Walter, H., Krob, E. J. & Tung, R. (1976) Hydrophobic affinity partition in aqueous two-phase systems of erythrocytes from different species. *Exp. Cell Res.* **102**, 14–24.
30. Biedler, J. L. & Riehm, H. (1970) Cellular resistance to actinomycin D in Chinese hamster cells *in vitro*: cross-resistance, radioautographic and cytogenetic studies. *Cancer Res.* **30**, 1174–1184.
31. Danø, K. (1972) Cross resistance between vinca alkaloids and anthracyclines in Ehrlich ascites tumor *in vivo*. *Cancer Chemother. Rep.* **56**, 701–708.
32. Simard, R. & Cassingena, R. (1969) Actinomycin resistance in cultured hamster cells. *Cancer Res.* **29**, 1590–1597.
33. Kessel, D., Botterill, V. & Wodinsky, I. (1968) Uptake and retention of daunomycin by mouse leukemic cells as factors in drug resistance. *Cancer Res.* **28**, 938–941.
34. Kessel, D. & Wodinsky, I. (1968) *In vivo* and *in vitro* uptake of actinomycin D by mouse leukemias as factors in survival. *Biochem. Pharmacol.* **17**, 161–164.
35. Inaba, M. & Johnson, R. K. (1977) Decreased retention of actinomycin D as the basis for cross-resistance in anthracycline-resistant sublines of P388 leukemia. *Cancer Res.* **37**, 4629–4634.
36. Bosmann, H. B. (1971) Mechanism of cellular drug resistance. *Nature* **233**, 566–569.
37. Kessel, D. & Bosmann, H. B. (1970) On the characteristics of actinomycin D resistance in L5178Y cells. *Cancer Res.* **30**, 2695–2701.
38. Bosmann, H. B. & Kessel, D. (1970) Altered glycosidase levels in drug-resistant mouse leukemias. *Mol. Pharmacol.* **6**, 345–349.
39. Carlsen, S. A., Till, J. E. & Ling, V. (1976) Modulation of membrane drug permeability in chinese hamster ovary cells. *Biochim. Biophys. Acta* **455**, 900–912.
40. Juliano, R. L. & Ling, V. (1976) A surface glycoprotein modulating drug permeability in chinese hamster ovary cell mutants. *Biochim. Biophys. Acta* **455**, 152–162.
41. Danø, K. (1973) Outward active transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim. Biophys. Acta* **323**, 466–473.
42. Chitnis, M. P. & Johnson, R. K. (1978) Biochemical parameters of resistance of an adriamycin-resistant subline of P388 leukemia to emetine, an inhibitor of protein synthesis. *J. Natl. Cancer Inst.* **60**, 1049–1054.