unknown mechanism and the microsomal pathway as shown in Fig. 2. Knowledge of the enzymatic mechanism of both pathways may lead to useful prodrugs with improved selectivity.

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REFERENCES

1. J. L. Au and W. Sadée, *Cancer Res.* **40**, 2814 (1980).
2. S. D. Harrison, E. P. Denine and D. Giles, *Cancer Treat. Rep.* **60**, 1394 (1979).
3. R. K. Johnson, B. T. Garibjanian, D. P. Houchens, I. Kline, M. R. Gaston, A. B. Syrkin and A. Goldin, *Cancer Treat. Rep.* **60**, 1335 (1976).
4. J. L. Au and W. Sadée, *Cancer Res.* **39**, 4289 (1979).
5. J. L. Au, A. T. Wu, M. A. Friedman and W. Sadée, *Cancer Treat. Rep.* **63**, 343 (1979).
6. T. Manunaka, Y. Minami, Y. Umeno, A. Yasuda, T. Sato and S. Fujii, *Chem. pharm. Bull., Tokyo* **28**, 1795 (1980).
7. A. T. Wu, J. L. Au and W. Sadée, *Cancer Res.* **38**, 210 (1978).
8. H. Fujita and K. Kimura, in *Progress in Chemotherapy (Antibacterial, Antivirial, Antineoplast)* (Ed. G. K. Daikos), Vol. 3, pp. 159–64. Athens Hellinic Society for Chemotherapy, Athens (1974).
9. R. Paul, M. Fluchaire and G. Collardeau, *Bull. Soc. chim. Fr.* 668 (1950).
10. *Regis Lab Notes*, No. 17, p. 76. Regis Chemical Co., Morton Grove, IL (1974).
11. A. M. Cohen, *Drug Metab. Dispos.* **3**, 303 (1975).
12. S. Ohira, S. Maeqawa, K. Watanabe, K. Kitada and T. Saito, *Proceedings of the Ninth International Congress of Chemotherapy, London, July, 1975* (Eds. K. Hellmann and T. A. Connors), Vol. 8, pp. 197–203. Plenum Publishing, New York (1976).
13. M. Yamayama, A. Moriyama, N. Unemi, S. Hashimoto and T. Suzue, *J. med. Chem.* **20**, 1592 (1977).
14. A. J. Lin, R. S. Benjamin, P. V. Rao and T. L. Loo, *J. med. Chem.* **22**, 1096 (1979).

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Studies on a mode of resistance to m-AMSA

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Studies on patterns and modes of drug resistance in animal tumors have provided useful information regarding determinants of drug responsiveness. Several factors have been implicated in resistance to a group of natural and synthetic products including anthracyclines, actinomycin D, vinca alkaloids and alkylaminoanthraquinones. Mouse leukemia cell lines selected for resistance to any of these agents tend to be cross-resistant to the others [1–6]. This common mode of drug resistance appears to involve an operational barrier to drug accumulation which may be an energy-dependent active outward transport process [7–13]. The broad cross-resistance pattern could, therefore, reflect the specificity of an active exodus process [8, 10, 11].

Table 1. Drug responsiveness patterns *in vivo**

Drug	Dose (mg/kg)	P388	P388/AMSA
Aclacinomycin	12	88	88
Actinomycin D	0.36	96	116
Adriamycin	4.5	125	37
m-AMSA	12	112	12
Daunorubicin	3	62	27
Ellipticine	60	86	4
Vincristine	1.2	108	96

* Animals received 10^6 cells on day 0 and were treated with specified drug levels on days 1, 5 and 9. Results are described in terms of increased life-span of tumor-bearing animals relative to untreated controls.

In this study, we have examined a subline of the P388 murine leukemia selected [14, 15] for resistance to the synthetic aridine *N*-[4-(9-acridinylamino)-3-methoxyphenyl]-methanesulfonamide (m-AMSA), an antitumor agent [16] which binds to DNA [17] causing protein-associated DNA strand breaks [18]. The cross-resistance pattern in P388/AMSA was unusual, suggesting a new mode of drug resistance.

Derivation of the adriamycin-resistant P388/ADR cell line, together with procedures for measurement of drug-induced promotion of survival of tumor-bearing animals are described in Refs. 1 and 2. The P388/AMSA cell line was obtained by serial intraperitoneal passage of P388 cells in BALB/c × DBA/2 mice treated intraperitoneally with 4 mg/kg m-AMSA on days 4–10. After nine transplant generations, animals received drug on days 1–7; at twenty-four transplant generations, the m-AMSA resistant subline was obtained. Further characterization of this cell line is shown in Table 1. Full details are described in Ref. 15.

Drug resistance patterns were determined by *in vivo* studies involving treatment with anti-tumor agents on days 1, 5 and 9 after intraperitoneal transplant of 10^6 cells. Drug levels employed (Table 1) were non-toxic to control animals and represented the highest tolerated drug levels with this schedule. Drug-promoted response is reported in terms of percent increased life-span (%ILS). If the mean time to death after transplant for untreated animals is 7 days, a

Table 2. Transport and binding studies

System	Drug	P388	P388/ADR	P388/AMSA
Intact cells*	DNR	174 ± 10	83 ± 5†	168 ± 9
Intact cells	AMSA	54 ± 6	21 ± 4†	45 ± 6
Intact cells + NaN_3, − glucose‡	DNR	182 ± 7	161 ± 9	167 ± 7
Intact cells + NaN_3, − glucose	m-AMSA	55 ± 5	41 ± 5	53 ± 5
Cell nuclei§	DNR	119 ± 7	54 ± 4†	38 ± 5†
Cell nuclei	m-AMSA	9.3 ± 1.7	4.2 ± 1†	3.3 ± 0.5†

* Cells were incubated for 10 min at 37° with labeled drugs in complete growth medium (1 mg/ml glucose). Units = pmoles drug/10^6 cells (average of five experiments ± S.D.).

† Significantly different from P388 values ($P < 0.01$).

‡ As for "intact cells" except that glucose-free medium containing 10 mM NaN_3 was used for incubations.

§ Nuclei were isolated after 10-min incubations of intact cells in complete growth medium. Units = pmoles drug/10^6 nuclei.

%ILS value of 200 means that treated animals had a mean survival of 21 days. Data shown in Table 2 represent mean values from two to ten experiments each involving ten mice.

Radioactive drugs were obtained from SRI International, Menlo Park, CA, via the Chemical Resources Section of the Developmental Therapeutics Program, Division of Cancer Treatment, NCI. m-AMSA was labeled on carbon 9 with ^{14}C (specific activity: 20 Ci/mole). Radiopurity was assessed with silica TLC using chloroform–methanol (8:2) and was found to be > 98%. Daunorubicin was labeled with ^{14}C on carbon 14 (31 Ci/mole); radiopurity was measured using silica TLC and chloroform–methanol–acetic acid (80:20:4) and was found to be > 96%. Both drugs were diluted with carrier so that a concentration of 0.3 μg/ml contained 50,000 counts per min per 2 μl.

Adriamycin-resistant (P388/ADR) and m-AMSA-resistant (P388/AMSA) sublines of the P388 murine leukemias were maintained in cell culture using Fischer's medium supplemented with 10% horse serum and 1 μM mercaptoethanol. Drug resistance was not altered by growth in culture in the absence of the selecting agents.

Uptake of labeled drugs was measured [9, 10] after 10-min incubations by which time steady-state intracellular levels had been reached. Isolation of cell nuclei was carried out by homogenization in hypotonic $CaCl_2$ [8] or citric acid [18].

The cross-resistance pattern observed in P388/AMSA was unusual. The line was found responsive to the anthracycline aclacinomycin, and to vincristine and actinomycin D, but was resistant to adriamycin and daunorubicin (Table 1). The P388/ADR cell line was resistant to all drugs named in Table 1, with %ILS values <20%, using the dose and schedule described in the table legend.

When 7 mg cell pellets were suspended in 1 ml of growth medium and incubated with 0.3 μg/ml levels of labeled daunorubicin (DNR) or m-AMSA, we observed impaired drug accumulation in P388/ADR but not in P388/AMSA (Table 2). Inhibition of energy-yielding metabolic processes by addition of sodium azide to glucose-free medium resulted in a marked potentiation of uptake of daunorubicin and m-AMSA in P388/ADR but not in P388/AMSA (Table 2). Such promotion has been attributed to the inhibition of energy-dependent drug exodus in P388/ADR [8, 9, 12, 18]. The combination of glucose deletion and NaN_3 addition was required for optimal uptake promotion. When uptake was examined in glucose-free medium *without* addition of NaN_3, drug accumulation by P388/ADR = 138 ± 9 pmoles/10^6 cells (daunorubicin); 36 ± 2 pmoles/10^6 cells (m-AMSA).

Isolation of nuclei from drug-loaded cells (Table 2) showed that impaired uptake in P388/ADR was associated with a corresponding decrease in nuclear drug binding. In contrast, the P388/AMSA cell line showed impaired nuclear drug penetration although net drug uptake was similar to that observed with P388 cells. These results were obtained using two procedures for nuclear isolation; data in Table 2 were derived via the citric acid method [19]. Conclusions drawn from this study depend on absence of drug translocation during nuclear isolation, i.e. the nuclear-bound fraction described in Table 2 represents only tightly bound drug.

Different anthracyclines show various affinities for the cell nucleus [20]; the correlation between cytoplasmic and nuclear drug concentration versus cytotoxicity has yet to be established. The data shown in Table 2 suggest that a barrier to nuclear uptake, rather than impaired cellular drug accumulation, accounts for resistance to m-AMSA in the P388/AMSA cell line. This result suggests to us a possible basis for the resistance pattern observed with P388/AMSA. Agents which can exert cytotoxic action from outside the nucleus may be toxic to this cell line; toxicity of drugs requiring nuclear accumulation may be diminished. This hypothesis suggests that the vinca alkaloids and actinomycin D can exert toxic action from extra-nuclear loci.

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REFERENCES

1. R. K. Johnson, M. P. Chitnis, W. M. Embrey and E. B. Gregory, *Cancer Treat. Rep.* **62**, 1535 (1978).
2. R. K. Johnson, A. A. Ovejera and A. Goldin, *Cancer Treat. Rep.* **60**, 99 (1976).
3. K. Dano, *Cancer Chemother. Rep.* **56**, 701 (1972).
4. T. Nishimura, K. Muto and N. Tanaka, *J. Antibiot., Tokyo* **31**, 493 (1978).
5. H. E. Skipper, D. J. Hutchison, F. M. Schabel Jr., L.

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H. Schmidt, R. W. Brockman, J. M. Venditti and I. Wodinsky, *Cancer Chemother. Rep.* **57**, 493 (1972).
6. L. J. Wilkoff and E. A. Dulmadge, *J. natn. Cancer Inst.* **61**, 1521 (1978).
7. M. P. Chitnis and R. K. Johnson, *J. natn. Cancer Inst.* **60**, 1049 (1978).
8. M. Inaba and R. K. Johnson, *Cancer Res.* **37**, 4629 (1977).
9. M. Inaba and R. K. Johnson, *Biochem. Pharmac.* **27**, 2123 (1978).
10. M. Inaba, H. Kobayashi, Y. Sukarai and R. K. Johnson, *Cancer Res.* **39**, 2200 (1979).
11. D. Kessel and I. Wodinsky, *Biochem. Pharmac.* **17**, 161 (1967).
12. T. Skovsgaard, *Cancer Res.* **40**, 1077 (1980).
13. M. Inaba, R. Fujikura and Y. Sakai, *Biochem. Pharmac.* **30**, 1863 (1981).
14. R. K. Johnson and W. S. Howard, *Proc. Am. Ass. Cancer Res.* **22**, 250 (1981).
15. R. K. Johnson and W. S. Howard, *Eur. J. Cancer clin. Oncol.*, in press.
16. B. F. Cain and G. J. Atwell, *Eur. J. Cancer.* **12**, 539 (1974).
17. M. J. Waring, *Eur. J. Cancer* **12**, 995 (1976).
18. R. S. Ungerleider, J. A. Zwelling, D. L. Glaubiger and K. Kohn, *Proc. Am. Ass. Cancer Res.* **21**, 295 (1981).
19. H. Busch, *Meth. Enzym.* **12A**, 421 (1967)
20. S. Seeber, H. Loth and S. T. Crooke, *J. Cancer Res. clin. Oncol.* **98**, 109 (1980).