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Effect of Dithiocarbanilates on Some Biological and Biophysical Properties of Leukemia L1210 Cell Membranes

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SUMMARY

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This study was designed to seek correlations between membrane alterations detected by transport studies and those measured by two biophysical techniques. A group of substituted dithiocarbanilates was found to alter selectively different membrane properties related to permeability and transport. We found a correlation between disruption of a membrane permeability barrier to accumulation of actinomycin D and an enhanced fluorogenic interaction between cells and a dansyl cadaverine probe. Inhibition of facilitated diffusion of nucleosides across the cell membrane was correlated with a more "hydrophobic" cell surface, detected by two-phase aqueous polymer partitioning studies in an "uncharged" system. Inhibition of active transport of a model amino acid, cycloleucine, was correlated with reduced cell-surface and membrane charge, detected by two-phase partitioning studies in a "charged" system. Dithiocarbanilates that caused inhibition of amino acid transport also quenched the fluorogenic cell interaction with dansyl cadaverine, suggesting a more generalized chaotropic drug effect. These findings suggest loci of membrane processes regulating permeability, and of barriers to substrate movement within the cell membrane.

INTRODUCTION

Since the cell membrane is such an important determinant of cell behavior, many procedures have been devised for measurement of biological and biophysical membrane characteristics. But the complexity of the membrane structure has made interpretation of data difficult. In the present study, we have examined effects of a series of drug analogues on different membrane properties. The latter include biological functions (maintenance of permeability barriers and transport processes) and biophysical properties (fluorogenic interactions and partitioning behavior in two-phase aqueous polymer systems). The correlations between different observations have provided clues concerning the loci of different membrane functions.

Agents which disrupt permeability barriers or transport systems can be identified by measuring rates of inward transport of different substrates. We previously described a membrane barrier to the permeation of the drug actinomycin D in murine leukemia cells (1). Several studies suggest that this barrier is associated with membrane glycoprotein components (1-3). Be-

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cause of its transport properties, actinomycin D is a useful probe for membrane permeability alterations. Another important membrane property is mediation of selective transport processes. To measure typical transport functions, we have utilized the nonmetabolized amino acid cycloleucine (4), along with metabolizable nucleosides (4, 5). Uptake of the latter was measured at low temperatures to minimize (6) subsequent intracellular metabolic processes. A nonmetabolizable nucleoside analogue, 5'-deoxyadenosine, has also been employed (7).

Among the more sensitive means for characterizing biophysical properties of the cell membrane is the technique of partition of cells in a two-phase aqueous polymer system (8, 9). Although this procedure has been mainly used to detect alterations in membrane properties associated with surface charge, other membrane properties, e.g., hydrophobicity (10, 11) can also be monitored.

Fluorescent probes have been widely employed to elucidate membrane characteristics. Although 1-anilinonaphthalene-8-sulfonate has been most extensively studied (12), we have employed the dye dansyl cadaverine (13), which appears to be a more specific probe for membrane alterations.

Using a series of dithiocarbanilates that selectively alter different biologic membrane properties, we have attempted here to correlate such effects with partitioning behavior and fluorogenic interactions.

MATERIALS AND METHODS

Organic synthesis. Preparation of the thiocarbanilates was carried out by R. S. McElhinney and C. N. Lucey; synthetic details will be published elsewhere.[1]

Chemicals. Sources and preparation of labeled actinomycin D, uridine, and cycloleucine are described in reference 4. Dextran T 500 (lot 7863) was provided by Pharmacia, polyethylene glycol (MW 6000) by Pierce Chemical Co., as was dansyl cadaverine.

[1] We acknowledge with thanks the gift of 3-aminophenylsulfur pentafluoride HCl, used in the preparation of compound 16, from Dr. W. A. Sheppard, E. I. Dupont de Nemours & Co., Wilmington, Delaware.

Cells and incubation procedures. L1210 murine leukemia cells, obtained from the Arthur D. Little Corp., Cambridge, Mass., were maintained in culture using MEM[2] supplemented with 10% fetal calf serum and gentamicin sulfate. Cells were obtained during exponential growth and then suspended in PBS or in fresh growth medium with HEPES (pH 7.4) replacing $NaHCO_3$ to permit brief incubations at high cell densities without affecting pH. Suspensions of 5×10^6 cells/ml were treated with 0.1 mM levels of different dithiocarbanilates for 10 min at 37°. The cells were then washed free from drug, using PBS, and resuspended in HEPES-buffered growth medium. Accumulation of labeled cycloleucine and actinomycin D was measured over a 5 min interval at 37°; accumulation of labeled uridine and 5'-deoxyadenosine was measured at 10° (0.3–5 min) as described in references 4 and 7.

Partitioning behavior of cells. After treatment of cells with drugs as described above, pellets containing 10^6 cells were suspended in 50 μl of 0.9% NaCl and then added to a 10-g mixture containing 5.0% (w/w) Dextran T 500 + 3.6% PEG (w/w) made up in 140 mM NaCl + 10 mM sodium phosphate buffer at pH 7.0, and containing 0.001% PEG-palmitate[3] (system 1). System 2 contained 5% Dextran and 3.6% PEG in 75 mM NaCl + 60 mM sodium phosphate buffer at pH 7. After gentle mixing of the phases plus cell suspension, a 1 ml portion was removed and the cell concentration measured with a Coulter Electronic Particle Counter, after appropriate dilution. The phases were then permitted to separate (20 min, 22°). An aliquot of the upper phase was removed and the cell number was measured as before. The partition coefficient is defined here as the number of cells present in the top phase expressed as the percent-

[2] Abbreviations used are: DMF, N,N'-dimethylformamide; PEG, poly(ethylene glycol) (mol wt 6,000); MEM, minimal-essential Eagle's medium (spinner modification); HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate; PBS, phosphate-buffered saline (130 mM NaCl-20 mM sodium phosphate (pH 7.4)); UR, uridine.

[3] Synthesized according to ref. 20; 55–60% of the hydroxyl groups were esterified.

age of the total number of cells (9). In reporting data, we show the relative partition coefficient as percentage of control; 100% representing the partition coefficient of untreated cells in a particular partitioning system.

Fluorescence studies. Drug-treated and control cells (10^7) were suspended for 5 min at 20° in 1 ml of PBS containing 10 μM dansyl cadaverine. Half of this suspension was then transferred to a quartz cuvette for determination of fluorescence (excitation, 340 nm; emission maximum, 500–520 nm). This provides a measure of the fluorescence (f) of bound dansyl cadaverine. To measure total bound dye (b), cells from the remaining half of the cell suspension were collected and homogenized in 1.5 ml of n-propanol. Fluorescence was then measured as described above (excitation, 340 nm; emission, 520 nm). The fluorescence yield, defined here as 100 (f/b), is a measure of the extent of interaction between bound dansyl cadaverine and fluorogenic sites (14).

Electrophoretic mobility studies. Cells were suspended in a medium containing 4.5% sorbitol, 14.5 mM NaCl and 0.6 mM $NaHCO_3$ at pH 7.2 and their electrophoretic mobility determined at 25° in a capillary tube using the Mark 2 Electrophoresis apparatus supplied by Rank Bros., Cambridge, England. At least 15 cells were used for each reported measurement, with reversal of polarity between determinations. A further description of the method is provided in reference 15.

RESULTS

Compounds tested. The dithiocarbanilates have the general structure:

$$R_1\text{—NH—}\overset{\overset{S}{\|}}{C}\text{—}SR_2.$$

All were soluble in DMF and such solutions were stable at −20°. Representative structures are shown in Fig. 1; a complete description of drugs tested is shown in Table 1.

Presentation of data. Numbers presented in Table 1 represent the average of at least 5 measurements. A variation of ±10% of these values was encountered, e.g.,

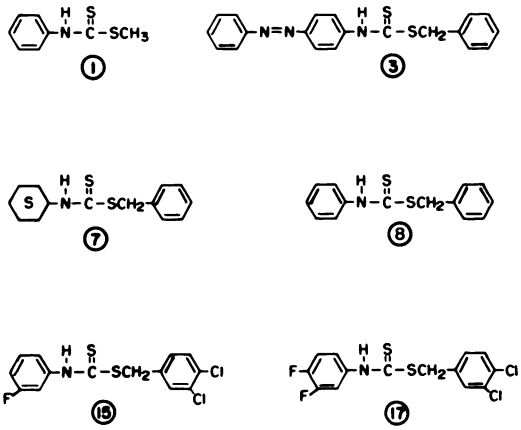

FIG. 1. *Structure of 6 dithiocarbanilates described in Table 1*

Circled numbers correspond to drug identification shown in Table 1 and Fig. 2.

a partition coefficient of 130 means (130 ± 13%) of control. All values are normalized to controls to simplify comparison of results. Specific results, in terms of counts/min of radioactive substrate in cell pellets, or percentage of total cell number found in the upper phase of partition systems is given below. Data obtained with radioactive uridine at 10° could be duplicated using the nonmetabolized nucleoside 5′-deoxyadenosine, indicating that the slow intracellular phosphorylation of the former was not a factor in results reported here. We have presented data on experiments involving uridine because of the familiarity of other investigators with this substrate.

Uptake studies. A summary of results is shown in Table 1. Rates of accumulation of actinomycin D, cycloleucine and uridine are shown as percentage of control (L1210 cells not treated with any drug). No more than 5 μl of drug solution in DMF was added per ml of cell suspension. This level of DMF did not alter any cell property measured.

Under conditions employed here, control cells accumulated actinomycin D from an extracellular concentration of 0.1 μg/ml such that a 7 mg cell pellet (wet weight) contained approximately 1,500 counts/min of radioactivity after a 5 min incubation. Accumulation was linear with respect to time for at least 30 min. Cycloleucine uptake was measured from an extracellular

TABLE 1

Effect of dithiocarbanilates on transport and partitioning behavior of L1210 cells

Rates of accumulation of actinomycin D (AD), uridine (UR), and cycloleucine (CL) were measured in drug-treated cells as described in the text, as was behavior of cell populations in 2-phase partitioning mixtures; (system 1) containing PEG-P (PC_1) or phosphate (system 2, PC_2). All data are shown as percentage of control after treatment for 10 min at 37° in medium containing 0.1 mM drug concentrations. These data represent the average of at least 5 determinations for each value; the variation was < ±10% of numbers shown.

Number	Structure	AD	UR	CL	PC_1	PC_2
1	*PhNHCSCH$_3$	100	100	100	90	95
1A	3,4-$Cl_2C_6H_3NH$—C(=S)—SCH_3	250	35	30	135	65
1B	3—$CF_3C_6H_4NH$—C(=S)—SCH_3	200	20	50	150	75
2	PhN(CH$_3$)—C(=S)—SCH_3	130	100	90	110	90
3	*PhN=NC_6H_4(4—)NHC(=S)—SCH_2Ph	200	90	85	115	100
3A	PhN=NC_6H_4(4—)NH—C(=S)—$SCH_2C_6H_4Cl$(4—)	185	90	100	115	100
4	PhN(CH$_3$)—C(=S)—$S(CH_2)_6CH_3$	205	100	100	110	100
4A	3,4—$Cl_2C_6H_3NH$—C(=S)—$S(CH_2)_5CH_3$	340	15	10	160	55
5	PhN(CH$_3$)—C(=S)—CH_2Ph	260	100	100	112	100
6	PhN(CH$_3$)—C(=S)—CH_2CH_3	270	50	85	135	85
7	*CyclohexylNHC(=S)—CH_2Ph	300	47	100	120	95
8	*$PhCH_2$NHC(=S)—CH_2Ph	320	25	75	130	90
9	3,4—$Cl_2C_6H_3$NHC(=S)—$CH_2C_6H_4NO_2$(4—)	230	5	45	190	70

TABLE 1—CONTINUED

Number	Structure	AD	UR	CL	PC_1	PC_2
9A	3,4—$Cl_2C_6H_3NH$—C(=S)—$SCH_2C_6H_4CH_3$(4—)	360	20	15	150	60
10	3,4—$Cl_2C_6H_3NHCSCH_2C_6H_3(OCH_2O)$(3,4—)	200	5	60	170	85
10A	3,4—$(CH_3)_2C_6H_3NH$—C(=S)—$SCH_2C_6H_3Cl_2$(3,4—)	205	55	95	130	95
11	$PhNHCSCH(CH_3)_2$	140	80	80	120	80
11A	3,4—$Cl_2C_6H_3NH$—C(=S)—$SCH(CH_3)_2$	380	5	5	195	50
12	$pHNHCSCH_2Ph$	200	40	50	120	75
13	$PhN(C_2H_5)$—C(=S)—$CSCH_3$	200	55	65	130	100
14	$PhNH$—C(=S)—$CH(CH_3)$—Ph	250	18	10	155	50
15	*3—$FC_6H_4NHCSCH_2C_6H_3Cl_2$(3,4—)	250	20	10	160	50
16	3—$SF_5C_6H_4NHCSCH_2C_6H_4Cl$(4—)	270	20	10	145	58
17	*3,4—$F_2C_6H_3NHCSCH_2C_6H_3Cl_2$(3,4—)	310	15	10	158	60
18	$PhNHCSCH_2Ph$	340	15	15	165	60
19	3—$CF_3C_6H_4NH$—C(=S)—$SCH_2C_6H_4Cl$(4—)	350	20	10	150	50

* Structures shown in Fig. 1.

In the structural notation for this table, Ph = C_6H_5-.

level of 0.1 mM; accumulation was linear for at least 15 min. A distribution ratio of 2.3 was achieved in control cells during a 5 min interval, representing 2,300 counts/min/ cell pellet. Uridine accumulation was measured from an extracellular pool of 20 μM. After 5 min, an apparent distribution ratio of 1.1 was achieved, representing 2,500

counts/min/cell pellet. To distinguish between transport and phosphorylation, we also studied transport of the nonmetabolized nucleoside 5'-deoxyadenosine (7) at 10–20° over 0.3–1 min intervals. The results did not differ significantly from those obtained with labeled uridine.

Structure-activity relations. Although the number of structural variations shown in Table 1 is not exhaustive, certain correlations can be noted. One group of compounds was inactive (#1), a second group promoted uptake of actinomycin D (#4), a third group promoted actinomycin D uptake and inhibited nucleoside transport (#7), while a fourth group resembled group 3 but also caused inhibition of cycloleucine transport (#15). Variation of R_2 is probably less significant that that of R_1. A halogen-substituted phenyl group at R_1 strongly promoted activity of agents with aliphatic substituents at R_2 (#1A). With regard to the R_1 position, substituents other than halogens on the benzene ring reduced activity. Certain peculiarities were noted, e.g., a $N{=}N{-}C_6H_5$ substituent on the phenyl group at R_1 abolished inhibition of nucleoside and amino acid transport (compare compounds 3 and 3A with 18). Cycloleucine transport inhibition was abolished by cyclohexyl at R_1 (#7) or by a methylene group interposed between N and phenyl (compare compounds 8 and 18).

Partitioning studies: Hydrophobic interactions. When L1210 control cells were partitioned between the phases of system 1, but in the absence of PEG-palmitate, less than 1% of the cells were found in the upper phase. This result was not significantly changed by any drug examined here. Incorporation of 0.001% PEG-palmitate markedly increased the number of cells partitioning into the upper phase. This represents an adequate concentration of the palmitate for interaction with palmitate receptors on the cell surface; further elevation of the palmitate concentration did not increase the number of cells that appeared in the upper phase.

In general, the number of cells which partitioned into the upper phase of system 1 was enhanced by prior treatment of cells with different dithiocarbanilates. Furthermore, we found a marked correlation between drug-induced inhibition of uridine transport and enhanced partition of cells into the upper phase of system 1 (Fig. 2, left). A logarithmic curve with the equation [% UR inhibition = 224 − 25.1 ln PC_1] was found to be significantly correlated with the points shown ($r^2 = 0.9$).

Partitioning studies: Charge-associated interactions. The use of a partitioning system containing a high level of phosphate buffer at pH 7.0 (system 2) permits the determination of charge-associated alterations in the cell membrane as a function of drug treatment (9). Under control condi-

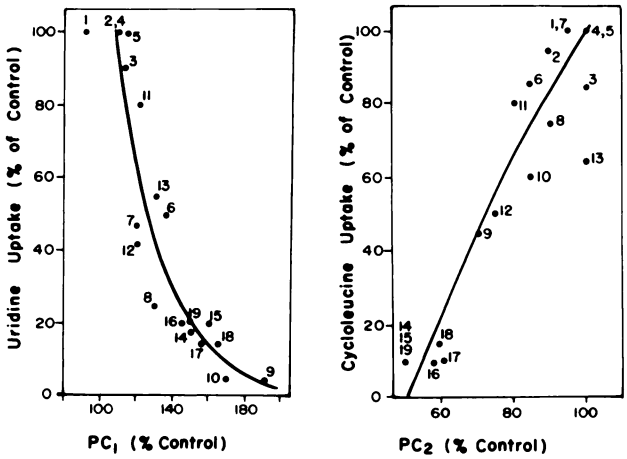

FIG. 2. *Left: Correlation between inhibition of uridine transport and partition coefficient in system 1 (hydrophobic interactions) of drugs defined in Table 1. Right: Correlation between inhibition of cycloleucine transport and partition coefficient of cells in system 2 (charge-associated interactions)*

All values shown are % of control.

tions, approximately 40–50% of the total number of cells partitioned into the upper phase of this polymer mixture. The data of Table 1 show that exposure of cells to certain dithiocarbanilates caused a reduction in this number, indicating a reduced negative charge on the cell membrane. A plot of relative inhibition of cycloleucine transport vs. partition of cells in system 2 (Fig. 2, right) showed a marked correlation. The curve shown represents an exponential plot with the equation

$$y = A\ e^{Bx}$$

where y = drug-induced change in partition coefficient (% of control), A = 51.7 and B = 0.00677 (values derived empirically), x = drug-induced inhibition of cycloleucine transport, expressed as percentage of control.

Electrophoretic mobility studies. Untreated L1210 cells had an electrophoretic mobility of −2.05 ± 0.10 μm/sec/V/cm. Treatment of cells with drugs 1 and 5 (Table 1) did not significantly alter this result. Electrophoretic mobility was lowered to −1.85 ± 0.07 by drug 8, to −1.10 ± 0.09 by drug 15, and to −1.22 ± 0.08 by drug 17.

Fluorescent dye studies. Effects of drugs listed in Table 1 on the fluorogenic interaction between L1210 cells and dansyl cadaverine were measured. Selected results are shown in Table 2. Inactive drugs did not alter the binding or fluorescence of dansyl cadaverine. Agents which only promoted actinomycin D uptake, e.g., drug 5, did not promote dansyl cadaverine uptake, but did promote the fluorescence yield of the bound dye, and blue-shifted the wavelength of maximum light emission. Agents that also inhibited nucleoside transport, e.g., drugs 6, 7, 8, and 10A, markedly promoted both uptake of dansyl cadaverine and fluorescence of bound drug, but the λ_{max} of emitted light was not further blue-shifted (drug 7 shown). Finally, agents which also inhibited amino acid transport, e.g., 14 and 18, also promoted dansyl cadaverine uptake, but the fluorescence was partly quenched and the blue-shift was not observed.

TABLE 2

Effect of selected dithiocarbanilates on fluorescence yield and emission λ_{max} of cells treated with dansyl cadaverine (DCV)

Cells were incubated for 10 min at 37° with 0.1 mM drug levels, then suspended in fresh PBS and treated with 10 μM dansyl cadaverine for 5 min at 20°. One portion of this suspension was used for fluorescence measurement (fluorescence and λ_{max} shown below). Cells from a duplicate portion were collected and suspended in propanol for the determination of total bound dansyl cadaverine. Results shown as mean ± S.D.

Drug[a]	Bound DCV[b]	Fluorescence[b]	Fluorescence yield[c]	λ_{max}
			%	
none	220 ± 15	100	45	512
1	220 ± 12	100 ± 9	45	512
5	220 ± 16	133 ± 12	66	500
7	290 ± 18	250 ± 17	86	500
14	140 ± 16	83 ± 10	34	515
18	225 ± 15	80 ± 9	35	515

[a] Structure shown in Table 1.

[b] Arbitrary units normalized such that fluorescence of control cells = 100.

[c] Fluorescence yield: $\frac{\text{fluorescence}}{\text{bound DCV}} \times 100$.

DISCUSSION

In the present study, we have employed a series of dithiocarbanilates to investigate the correlations between several methods used for detection of membrane alterations. Structure-activity relations, outlined above, are not the major concern of this report, but they indicate the marked sensitivity of membrane effects to minor variations in drug structure.

Of the experiments described here, the transport studies are the least ambiguous. The lipophilic drug actinomycin D penetrates the L1210 cell poorly; the barrier to drug uptake is apparently predominantly glycoprotein (1–3). Disruption of this barrier by surface-active agents (4, 16, 17) can markedly promote the rate of actinomycin D uptake by L1210 cells. We have therefore employed radioactive actinomycin D as a probe for disruption of a membrane permeability barrier.

Facilitated diffusion (e.g., of nucleosides) and active transport (e.g., of amino acids) were monitored by the use of uridine (at a low temperature to minimize subsequent phosphorylation) and the nonmetabolized amino acid cycloleucine. We later repeated experiments involving the former com-

pound with the nonmetabolized nucleoside 5'-deoxyadenosine (7) so that transport, totally delineated from subsequent phosphorylation, could be measured. Both facilitated diffusion and active transport are presumably mediated via integral membrane proteins (18); interference with structure and/or function of these proteins would therefore affect movement of their transport substrates into the cell.

With regard to the biophysical studies, the two-phase partitioning systems have been extensively used to gain information regarding cell surface properties (4, 9–11, 19). In the presence of a high phosphate/chloride ratio, the number of cells that partition into the upper phase is a function of the net charge on the cell surface (8, 9, 19). With this procedure, charge-associated alterations can be detected by a procedure considerably more rapid, and involving a larger number of cells, than can be measured by microelectrophoresis studies of individual cells. But direct measurement of electrophoretic mobility using a group of 5 drugs provided data that correlated with results obtained with partitioning system 2, i.e., agents that reduced partition of cells into the upper phase also decreased the net negative cell charge.

Another partitioning system involves the use of an 0.001% level of PEG-palmitate in a mixture with a low phosphate/chloride ratio (9–11, 20). In the absence of the palmitate, few cells partition into the upper phase, since the electrostatic potential between the phases is small (19). The palmitate preferentially partitions into the PEG-rich upper phase, bringing with it any cell with a sufficient number of surface palmitate receptors, presumably hydrophobic in nature. The relative number of cells appearing in the upper phase of a mixture containing PEG-palmitate is therefore considered to be a reflection of the relative number of such "hydrophobic" surface receptors. We define here "hydrophobicity" as the relative number of such cell-surface receptors.

A variety of fluorescent probes have been used to examine the environment of different regions of the cell surface. We have employed here the fluorogenic dye dansyl cadaverine, which may have a preferential affinity for membrane regions (13). The specificity of this probe for the membrane has recently been questioned (2), but dansyl cadaverine has two properties useful in studying drug-membrane interactions: the wavelength of maximum emission is blue-shifted as the environment of the probe becomes more hydrophobic, and the fluorescence yield is not enhanced by most proteins but is enhanced by anionic phospholipids (13). We have measured total uptake of dansyl cadaverine by cells, the fluorescence yield of the bound dye, and the emission λ_{max}.

We found several dithiocarbanilates capable of promoting actinomycin D uptake with minimal effects on transport. These compounds did not alter partitioning of cells in the "charged" system, but slightly enhanced the partition coefficient of cells in a two-phase system designed to detect alterations in surface hydrophobicity. Uptake of the fluorescent probe dansyl cadaverine was not altered by such drugs (e.g., compound 5, Table 1), but the fluorescence yield was promoted (Table 2) and the emission λ_{max} was shifted toward the blue. These data are consistent with the supposition that drugs that disrupt a barrier to actinomycin D expose lipid-rich membrane regions to the environment. Our data therefore support a hypothesis that agents that enhance access of hydrophobic lipid sites on the membrane to extracellular interactions will promote uptake of actinomycin D and similar lipophilic drugs.

Drugs that both increased uptake of actinomycin D and inhibited nucleoside transport caused an increase in the partition coefficient of cells in a two-phase system designed to detect membrane alterations not associated with surface charge. A striking correlation between percentage of inhibition of nucleoside transport and partition coefficient was observed (Fig. 2, left). We propose that a population of hydrophilic surface receptors involved in nucleoside transport is disrupted by exposure to appropriate dithiocarbanilates, such that cell-surface hydrophobicity is increased and nucleoside transport is inhibited. When L1210 cells were exposed to such drugs (e.g., compound 7, Table 1), total binding of dansyl cadaverine was enhanced, and the fluo-

rescence yield was markedly enhanced (Table 2), while the blue-shift of emitted light was similar in extent to that produced by agents which caused only promotion of actinomycin D uptake. These data suggest that creation of an increasingly more hydrophobic cell surface has resulted in increased accumulation of a hydrophobic fluorescent dye. The enhanced fluorescent yield is consistent with the supposition that membrane structures responsible for nucleoside transport may mask hydrophobic membrane components. It should be emphasized at this point that the effects seen in this study may represent interactions between drugs and a wide variety of membrane components, of relatively few of which are involved in transport phenomena.

When we studied dithiocarbanilates that disrupted barriers to actinomycin D uptake and inhibited both transport of nucleosides and cycloleucine, a different pattern of biophysical properties was observed. The extent of inhibition of cycloleucine transport was found correlated with charge-associated (9, 20) partitioning behavior (Fig. 2, right). Furthermore, these dithiocarbanilates quenched the fluorescence yield of bound dansyl cadaverine, and the blue-shift seen with certain other drugs was abolished (Table 2). We propose that dithiocarbanilates showing these effects are strongly chaotropic, nonselectively disorganizing functions of the cell membrane. We found a similar pattern of drug effects when we examined a polyene antibiotic with strong surface-active properties (22).

Results described here show that the use of agents that selectively disrupt specific membrane functions can aid in interpretation of biophysical measurements of membrane phenomena. It seems likely that agents that disrupt specific hydrophilic barriers at the cell surface can promote binding and fluorescence yield of probes for hydrophobic membrane sites, e.g., dansyl cadaverine, and also promote affinity of cells for hydrophobic polymer phases. In the present study, the observed correlation between inhibition of amino acid transport and decreased membrane charge, detected by partitioning behavior, suggests that such transport may be associated with a charged membrane region. But this partitioning phenomenon might also be mediated by a nonspecific "chaotropic" drug effect, producing a multiplicity of membrane function disruptions. Experiments involving multiple detection systems appear to offer substantial aid in interpretation of results, as noted above.

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