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MODULATION OF MEMBRANE DRUG PERMEABILITY IN CHINESE HAMSTER OVARY CELLS

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SUMMARY

The kinetics of colchicine uptake into Chinese hamster ovary cells have been investigated and found to be consistent with an unmediated diffusion mode. A variety of compounds such as local anesthetics and non-ionic detergents as well as drugs such as vinblastine, vincristine, daunomycin and actinomycin D potentiate the rate of colchicine uptake into these cells and into colchicine resistant mutants. In all cases, higher concentrations of these compounds were required to stimulate colchicine uptake in the colchicine resistant mutants than in the cells of the parental line. This stimulation was observed also in the uptake of puromycin, a structurally and functionally different drug. These stimulatory agents did not, however, cause the cells to become nonspecifically leaky since the uptake of 2-deoxy-D-glucose was unaffected. In addition, the activation energy of colchicine uptake was unaltered in the presence of stimulating agents, implying that they were not causing colchicine to enter the cells via a different mechanism. The results are compatible with the view that these compounds are membrane-active, and are able to stimulate an increased rate of unmediated diffusion of colchicine into the cells. It appears that a mechanism for the regulation of passive permeability is modified in the resistant mutants.

INTRODUCTION

An important function of the plasma membrane is its role as a permeability barrier, yet little is known about the regulation of membrane permeability in eukaryotic cells. One approach to this problem is through the use of drug-resistant mutants. Previous studies of colchicine-resistant (CH^R) mutants of Chinese hamster ovary cells have indicated that resistance to the cytotoxic effects of colchicine is the result of a reduced permeability of the drug [1]. This reduced permeability is not restricted to colchicine, but affects the sensitivity of the cells to a wide variety of drugs, some structurally and functionally quite unrelated to colchicine [1, 2].

Some factors which modulate this drug permeability barrier have already been investigated. Metabolic inhibitors such as cyanide, azide and dinitrophenol enhance permeability [3]; this enhancement could be prevented by metabolizable

sugars such as glucose or ribose. It appears that the metabolic energy level is important in maintaining the permeability barrier. The non-ionic detergent Tween 80 also causes dramatic changes in colchicine uptake by Chinese hamster ovary cells [1].

In order to investigate further the nature of the drug permeability barrier in mammalian cells, we studied the kinetics of colchicine uptake into whole cells, and the effects of a variety of membrane active agents on this uptake in parental and drug-resistant mutants of Chinese hamster ovary cells.

MATERIAL AND METHODS

Chemicals and reagents

Colchicine, puromycin-HCl, procaine, DL-propranolol, Tween 80 and desiccated firefly lanterns were obtained from Sigma Chemical Co.; vinblastine sulfate (VELBE) from Eli Lilly and Co., adriamycin from Adria Laboratories Inc.; colcemid from CIBA; tetracaine from Schwarz/Mann.; xylocaine (Lidocaine) from Dr. R. Fynes, Astra Chemicals Co.; daunomycin (Cerubidine) from Poulenc Ltd.; actinomycin D (Cosmegen) from Merck, Sharp and Dohme; Triton X detergents from Rohm and Haas; [^{3}H]puromycin and 2-deoxy-D-[^{3}H]glucose from Amersham/Searle. [^{3}H]Colchicine (18 Ci/mmol) dissolved in benzene/ethanol was obtained from New England Nuclear. The solvent was removed in the dark under a stream of nitrogen and the [^{3}H]colchicine resuspended in phosphate buffered saline. All other chemicals were of reagent grade.

Cell lines and culture conditions

All the colchicine resistant lines [1] used in this investigation were derived from the glycine, adenosine and thymidine requiring auxotroph AUX B1 [4]. Six different colchicine resistant mutants were used in this study. These mutants fall into two sets (CH^R2H, CH^R2HA, CH^RC4 and CH^RA3, CH^RB3, CH^RC5) representing two independent three-step selections [1]. Procedures for maintaining mutant cell lines in culture were as described by Thompson and Baker [5]. The cells were grown in suspension culture at 37 °C in α-minimum essential medium [6] supplemented with antibiotics and 10 % fetal calf serum (Flow Laboratories).

Uptake measurements

Colchicine uptake was measured as previously described [3] using a final concentration of 1 μM colchicine except where otherwise indicated. In all experiments the drugs were added at the same time as the [^{3}H]colchicine. Uptake of [^{3}H]puromycin was performed as for colchicine using a final concentration of 1 μM puromycin.

Uptake of 2-deoxy-D-glucose was measured using cells growing in 35 mm plastic dishes (Linbro Chemical Co.). The cells were incubated with 2 μM 2-deoxy-D-[^{3}H]glucose (4.6 Ci/mmol) for various times, after which the dishes were rinsed three times with ice-cold phosphate buffered saline. The cells were then dissolved in 0.1 M NaOH and aliquots taken for protein determination [7] and for measurement of radioactivity.

ATP measurements

ATP levels were measured using the firefly luciferase assay as described by Stanley and Williams [8] using a Mark I Liquid Scintillation counter (Nuclear

Chicago). Cell extracts were prepared by adding 1 ml of cell suspension at $3 \cdot 10^6$ cells/ml to 0.5 ml of ice-cold 1.2 N perchloric acid. The precipitate was removed by centrifugation and the amount of ATP in 50 μl aliquots of this extract was assayed.

RESULTS

Colchicine uptake

At the outset of this study we were interested in determining the mechanism by which colchicine permeates Chinese hamster ovary cells, that is whether colchicine enters the cells via a mediated or an unmediated mode. Towards this objective, we examined several facets of colchicine transport, viz. (a) kinetics of colchicine uptake, (b) competition of uptake by a close structural analogue of colchicine, colcemid, and (c) effect of inhibitors of various mediated transport systems on colchicine uptake. These are described below.

The initial rate of colchicine uptake into Chinese hamster ovary cells was measured at varying colchicine concentrations from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ M as shown in Fig. 1 in the form of a Lineweaver-Burk plot. It can be seen in Fig. 1 that colchicine permeation exhibits nonsaturation kinetics consistent with an unmediated uptake mechanism. It should be noted however, that initial attempts to measure the rates of colchicine uptake at substantially higher drug concentrations (e.g. 10^{-3} M or higher) have met with technical difficulties associated with the washing procedures and the saturation of the colchicine binding sites in the cell.

Additional evidence that colchicine is taken up by an unmediated process is shown in Table I, where colcemid, a close structural analogue of colchicine, did not

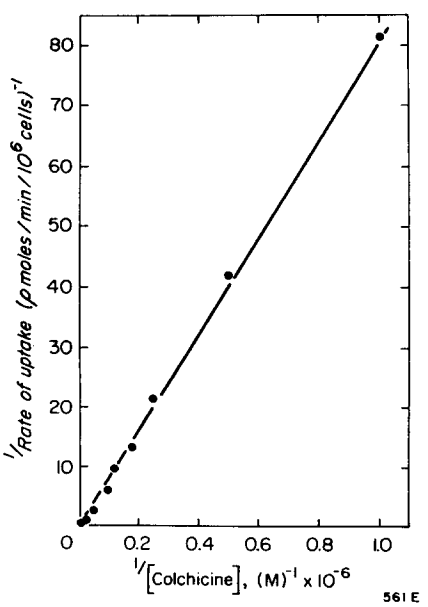

Fig. 1. Double reciprocal plot of rate of colchicine uptake versus colchicine concentration. Rates of uptake were determined from the linear portion of uptake versus time curves as shown in Fig. 2.

TABLE I

EFFECT OF COLCEMID ON COLCHICINE UPTAKE

Rate of colchicine uptake was measured from the linear portion of uptake versus time curves as shown in Fig. 2. The colcemid was added at the same time as the [^{3}H]colchicine.

Concentration of colcemid (M)	Rate of uptake (pmol/min per 10^6 cells)
0	0.016
$1.0 \cdot 10^{-6}$	0.017
$1.0 \cdot 10^{-5}$	0.013
$1.0 \cdot 10^{-4}$	0.025

inhibit the rate of uptake of colchicine at even 100-times the concentration of colchicine used.

The sulfhydryl reagents *p*-chloromercuribenzoic acid and *p*-chloromercuriphenylsulfonic acid as well as the cardiac glycoside ouabain, which are potent inhibitors of various mediated transport systems [9], did not inhibit colchicine uptake (data not shown). These data thus support the hypothesis that colchicine is taken up in Chinese hamster ovary cells by an unmediated process.

Effect of vinblastine on colchicine uptake

In testing the effect on colchicine uptake of various drugs to which the colchicine resistant cells are cross-resistant [1, 2], we found that one of these drugs, vinblastine, stimulated the uptake of colchicine in both the parental and mutant cell lines as seen in Fig. 2. The rates of colchicine uptake in the parental cell line AUX B1, and the highly colchicine resistant mutant CH^{R}C4, were 20 and 0.2 fmol/min per 10^6 cells respectively. However, upon the addition of 0.10 mM vinblastine, the rates of colchicine uptake increased to 160 fmol/min per 10^6 cells for the parental cell line and 150 fmol/min per 10^6 cells for the CH^{R}C4 cell line. It should be noted that when the cells were fully stimulated, the rates of colchicine uptake in the mutant and

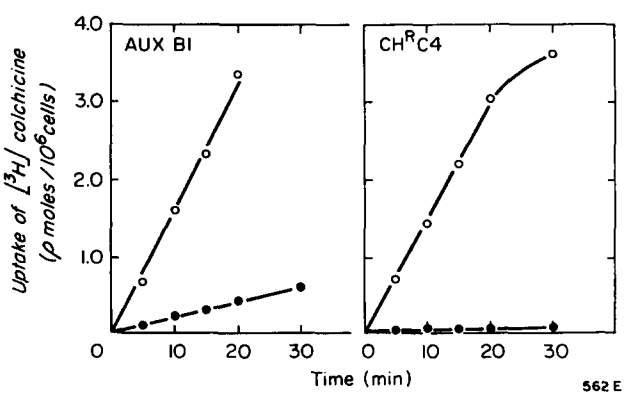

Fig. 2. Stimulation of colchicine uptake by vinblastine. The uptake of [^{3}H]colchicine was measured in the presence, (○); and absence, (●); of 0.1 mM vinblastine.

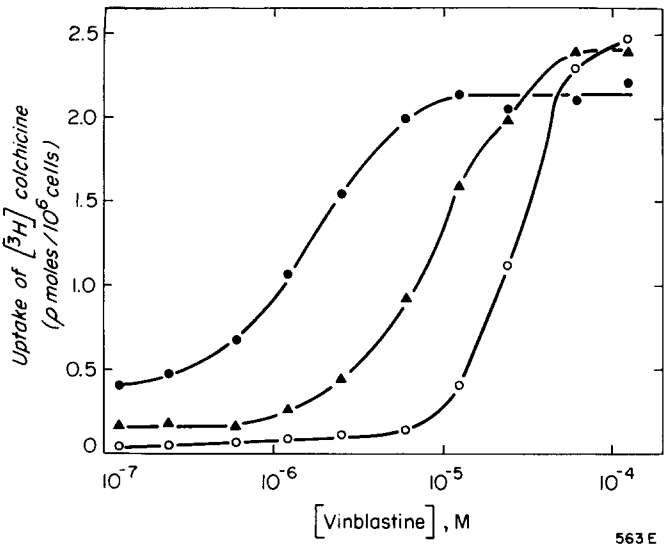

Fig. 3. Concentration dependence of stimulation of colchicine uptake by vinblastine. The amount of [^{3}H]colchicine taken up in 15 min at 37 °C was measured for AUX B1, (●); CH^{R}A3, (▲); and CH^{R}C4 (○).

parental cell lines became similar while in the unstimulated state they differed by about 100-fold. We have previously observed a similar effect when colchicine uptake was stimulated by metabolic inhibitors [3].

In order to define the concentration of vinblastine at which maximum colchicine stimulation is observed, the initial rates of colchicine uptake were measured as a function of vinblastine concentration (Fig. 3). The parental cell line AUX B1 required

TABLE II

CONCENTRATIONS OF VINBLASTINE REQUIRED TO STIMULATE VARIOUS CH^R-MUTANTS

Concentrations of vinblastine required to give 50 % maximum stimulation was determined from dose-response curves as shown in Fig. 3. Values for relative resistance of mutants to colchicine and vinblastine were taken from ref. 1.

Cell Line	Concentrations (M) of vinblastine to give 50 % maximum stimulation	Relative resistance	
		to colchicine*	to vinblastine**
AUX B1	$1 \cdot 10^{-6}$	1	1
CH^{R}2H	$3 \cdot 10^{-6}$	2	4
CH^{R}2HA	$7 \cdot 10^{-6}$	10	13
CH^{R}A3	$8 \cdot 10^{-6}$	12	7
CH^{R}B3	$2 \cdot 10^{-5}$	20	17
CH^{R}C4	$3 \cdot 10^{-5}$	88	58
CH^{R}C5	$4 \cdot 10^{-5}$	160	83

* Correlation coefficient, $r = 0.94$, $P < 0.01$.
** Correlation coefficient, $r = 0.96$, $P < 0.001$.

about 10^{-5} M vinblastine to yield maximum stimulation while the highly resistant mutant CH^RC4 required a 10-fold higher concentration. CH^RA3, a mutant of intermediate resistance required an intermediate concentration to give maximum stimulation. Thus it appears that there is a correlation between the amount of vinblastine required to stimulate colchicine uptake and the relative drug resistance of the cells. This is more rigorously documented in Table II with a number of independent colchicine resistant lines.

It is perhaps significant that mutant and parental cell lines were stimulated to about the same maximum level (Fig. 3); this level may represent a stable, highly permeable state of the cell membrane.

Since both colchicine and vinblastine interact with tubulin, the subunit of microtubules [10, 11], it was possible that vinblastine was altering colchicine uptake by affecting colchicine binding. To test this, the effect of vinblastine on the uptake of another drug, puromycin, to which the mutants are also cross-resistant but which is not thought to interact with microtubules was studied. As can be seen in Fig. 4, puromycin uptake was also stimulated at concentrations of vinblastine which caused similar stimulation of colchicine uptake; furthermore the concentration of vinblastine required to stimulate puromycin uptake in CH^RC4 was about 20-fold greater than that required to stimulate AUX B1. This result is consistent with the results obtained for the stimulation of colchicine uptake by vinblastine (Fig. 3 and Table II).

Since we had previously shown that metabolic inhibitors can stimulate colchicine uptake in these cells [3] we investigated whether vinblastine was affecting the ATP level resulting in a stimulation of drug uptake. Even 10 min after the addition of vinblastine the ATP level was not significantly reduced (2.17 and 2.16 nmol ATP/10^6

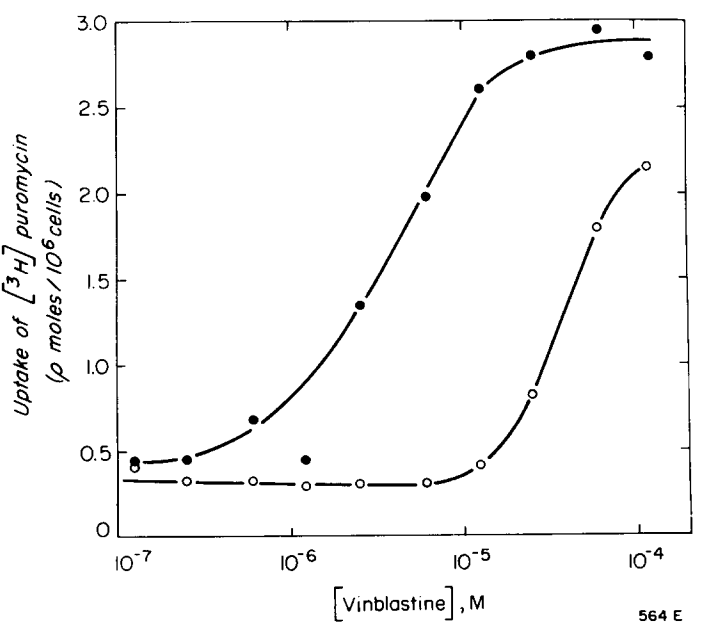

Fig. 4. Concentration dependence of stimulation of puromycin uptake by vinblastine. The amount of [^{3}H]puromycin taken up in 15 min at 37 °C was measured for AUX B1, (●); and CH^RC4, (○).

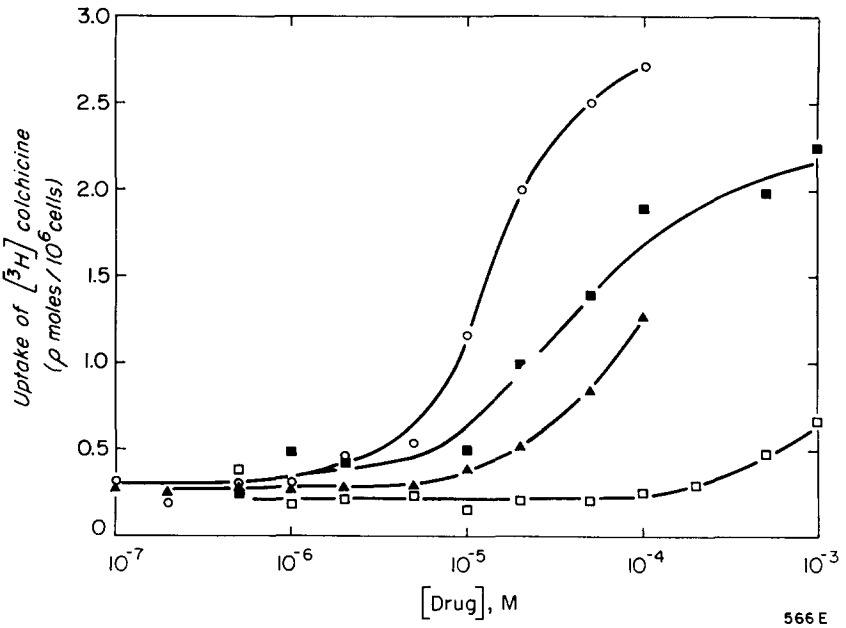

Fig. 5. Stimulation of colchicine uptake in AUX B1 by chemotherapeutic drugs. Amount of [^{3}H]-colchicine taken up in 15 min at 37 °C was measured. Drugs used were, vincristine, (○); daunomycin, (■); adriamycin, (□); and actinomycin D (▲).

cells in the presence and absence of vinblastine respectively). Moreover, in studies with metabolic inhibitors, stimulation of drug uptake was detected only in cells with ATP levels of less than 1.5 nmol/10^6 cells (Carlsen, S. A., unpublished). This implies that vinblastine did not cause stimulation of colchicine uptake by affecting intracellular ATP levels.

Previously Fyfe and Goldman [12] have found that vincristine, a structural analogue of vinblastine, increased the steady-state level of methotrexate in Ehrlich ascites tumor cells. They proposed however that this drug was acting as a metabolic inhibitor and thereby leading to stimulation. Thus their system seems to be significantly different from ours.

Since vinblastine is a widely used antineoplastic agent, other chemotherapeutic drugs were tested for their ability to potentiate drug uptake. Vincristine, daunomycin and actinomycin D potentiate colchicine uptake in Chinese hamster ovary cells (Fig. 5). Adriamycin, a drug structurally similar to daunomycin, did not affect colchicine uptake over the concentration range of $5 \cdot 10^{-7}$ to 10^{-3} M. Certain drugs which are different from each other both structurally and functionally were able to stimulate colchicine uptake in these cells while others were not.

Effect of local anesthetics and non-ionic detergents on colchicine uptake

Many compounds are known to interact with membranes; for example, Seeman et al. [13–15] have shown that local anesthetics and non-ionic detergents can interact directly with the membrane of human erythrocytes leading to protection against hypotonic hemolysis. Therefore, it was of interest to examine the effects of

TABLE III

CONCENTRATIONS OF LOCAL ANESTHETICS AND DETERGENTS REQUIRED FOR STIMULATION OF COLCHICINE UPTAKE

Concentration of compound required to give 50 % maximum stimulation was determined from dose-response curves as shown in Fig. 3.

Drug	Concentration of drug to give 50 % maximum stimulation	
	in AUX B1	in CH^RC4
Procaine	$1 \cdot 10^{-2}$ M	$3.5 \cdot 10^{-2}$ M
Lidocaine	$5 \cdot 10^{-3}$ M	$3 \cdot 10^{-2}$ M
Tetracaine	$4 \cdot 10^{-4}$ M	—
Propranolol	$5 \cdot 10^{-4}$ M	$1 \cdot 10^{-3}$ M
Tween 80	4 μg ml^{-1}	15 μg ml^{-1}
Triton X-45	4 μg ml^{-1}	15 μg ml^{-1}
Triton X-100	3 μg ml^{-1}	20 μg ml^{-1}
Triton X-102	2 μg ml^{-1}	20 μg ml^{-1}
Triton X-305	10 μg ml^{-1}	100 μg ml^{-1}

these "membrane active" agents on colchicine uptake. As can be seen in Table III, the local anesthetics potentiate colchicine uptake in a manner similar to that of vinblastine and at concentrations similar to those required for protection of erythrocytes against hypotonic hemolysis [13–15]. Higher concentrations of anesthetic were required to stimulate colchicine uptake in the mutant CH^RC4 than in the parental cell line AUX B1 even though the CH^R mutants are collaterally sensitive to the cytotoxic effect of these compounds [2]. This implies that the cytotoxicity of these compounds is a result of a different mechanism of action from that leading to stimulation of colchicine uptake.

Non-ionic detergents, which also fall into the group of membrane active compounds that can protect erythrocytes from hypotonic hemolysis [14, 15], could also stimulate colchicine uptake as seen in Table III. Thus we have found that many membrane active agents have the ability to stimulate colchicine uptake in these cells, and in general the colchicine resistant mutants required higher concentrations in order to stimulate drug uptake than did the parental cell line AUX B1.

To determine if the stimulatory agents were simply causing the cell to become non-specifically leaky, we tested the effect of vinblastine and Tween 80 on the uptake of 2-deoxy-D-glucose, a non-metabolized glucose analogue as seen in Fig. 6. We found that the rate of 2-deoxy-D-glucose uptake was virtually unaltered by the addition of vinblastine (0.51 cpm/μg protein per s compared to 0.47 cpm/μg protein per s in the presence of 0.10 mM vinblastine). The addition of Tween 80 however, caused a slight inhibition (0.35 cpm/μg protein per s). This supports the view that these agents do not cause a complete loss of the selective permeability barrier but may only increase the uptake of relatively hydrophobic agents entering by an unmediated process.

This evidence against non-specific disruption of the permeability barrier is in agreement with previous results where we showed that Tween 80, at concentrations up to 100 $\mu g \cdot ml^{-1}$, did not reduce the plating efficiency of the parental cell line or any of the mutant colchicine resistant lines [1]. At concentrations of detergent which

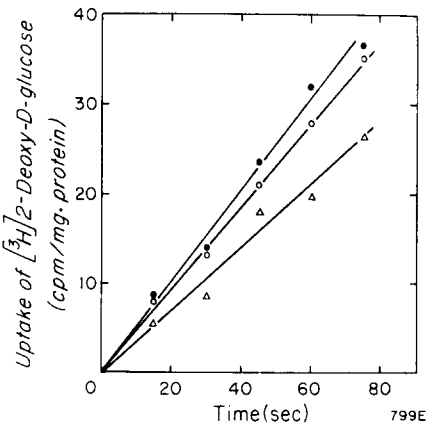

Fig. 6. Effect of vinblastine and Tween 80 on 2-deoxy-D-glucose uptake. The uptake of 2-deoxy-D-[^{3}H]glucose was measured in the absence, (●); or presence of 0.1 mM vinblastine, (○); or 50 μg ml^{-1} Tween 80, (△).

could cause a 6-fold increase in drug uptake of the parental cell line, and over a 20-fold increase in drug uptake of the mutant lines, the cells showed no apparent decrease in growth rate.

Drug uptake as a function of temperature

In order to determine if colchicine was taken up by the same mechanism in

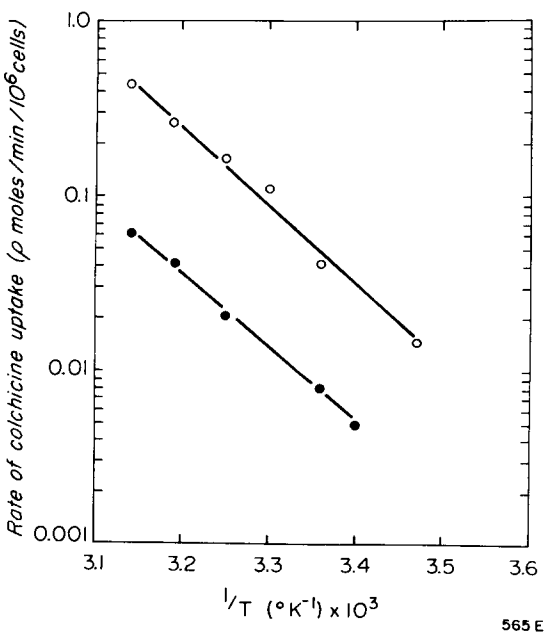

Fig. 7. Arrhenius plot of colchicine uptake in AUX B1. Rates of uptake were measured from the linear portions of uptake versus time curves as shown in Fig. 2.

both the presence and absence of vinblastine, we looked at the effect of temperature on colchicine uptake. As can be seen in Fig. 7, when the rate of colchicine uptake was measured in the presence and absence of a stimulating concentration of vinblastine at various temperatures, the temperature response was the same under both conditions. From the slope of the Arrhenius plot in Fig. 7, the activation energy for colchicine uptake was calculated to be 19 kcal · mol^{-1} while in the presence of vinblastine an activation energy of 20 kcal · mol^{-1} was found. This result is consistent with the hypothesis that the mechanism of uptake is the same in both the presence and absence of vinblastine (the stimulated and unstimulated states) and that it is only the rate of uptake which is altered.

DISCUSSION

Several lines of evidence indicate that colchicine permeates Chinese hamster ovary cells by an unmediated process. (a) The uptake of colchicine exhibits non-saturation kinetics (Fig. 1). (b) Colcemid, a close structural analogue of colchicine, does not compete with colchicine uptake (Table I). (c) Sulfhydryl reagents such as *p*-chloromercuriphenylsulphonate and *p*-chloromercuribenzoate at concentrations which inhibit glucose transport other mediated transport systems [9] do not affect colchicine uptake. (d) Colchicine uptake is greatly stimulated by detergents, local anesthetics, (Table III), and a number of amphipathic drugs (Figs. 2, 3 and 5). This stimulation was not due to a general leakiness of the cell membranes to low molecular weight compounds since the presence of a stimulating concentration of vinblastine did not alter the rate of uptake of 2-deoxy-D-glucose (Fig. 6). (e) In addition, the activation energy of colchicine uptake in these cells was unaltered in the presence of vinblastine (Fig. 7) implying that colchicine is taken up by the same mechanism in the presence and absence of stimulating agents. All these properties are consistent with the hypothesis that colchicine enters cells by an unmediated diffusion mechanism.

The potentiation of colchicine uptake by non-ionic detergents, local anesthetics and certain amphipathic drugs (Table III, Figs. 2, 3 and 5) provides a possible approach to elucidation of the mechanism of drug permeability. Seeman [13–16] has shown that a number of compounds, including the above-mentioned agents able to stimulate colchicine uptake, possess the common property of protecting erythrocytes from hypotonic hemolysis. The mechanism of this protection is thought to be mediated through an interaction of these compounds with the erythrocyte membrane leading to an expansion of the membrane [15]. Since similar concentrations of local anesthetics and vinblastine are required both for erythrocyte protection [13–16] and drug stimulation (Tables II and III), it seems likely that these compounds interact directly with Chinese hamster ovary cell membranes in a similar manner leading to the stimulation of drug uptake. Two observations suggest that this interaction results in increased membrane fluidity. First, in a number of model systems, local anesthetics have been shown to interact with membrane lipids increasing membrane fluidity [17–19]. Second, it is well documented that an increase in passive permeability of a number of different compounds is a consequence of increased membrane fluidity [20, 21]. The fact that in this present study we observed stimulation of colchicine and puromycin uptake by local anesthetics, non-ionic detergents and some amphipathic drugs, while at the same time no stimulation was observed in erythritol up-

take (unpublished data), a presumed passively diffusing compound, raises the possibility that the putative increased membrane fluidity induced by the above membrane active compounds may be restricted only to specific membrane domains [22].

Studies on the effects of these membrane active compounds on a number of independent mutants lend further support to this hypothesis. It is seen that irrespective of whether the mutants were cross-resistant (e.g. vinblastine) or collaterally sensitive (e.g. local anesthetics and certain detergents) [2] to these membrane active agents, in each case, increased amounts of these agents relative to the parental cell line were required to potentiate the rate of colchicine uptake in the mutant cells. These observations are consistent with the concept that certain domains of the mutant membranes are less fluid (reduced drug permeability), and that higher concentrations of the membrane active agents are required to increase the fluidity of these domains to the same level as that observed in the parental cell line.

Previously we have demonstrated that the drug permeability of Chinese hamster ovary cells was greatly increased in the presence of different metabolic inhibitors [3] and that this increased permeability was directly related to the reduced amount of intracellular ATP (Carlsen, S.A., unpublished). The membrane active agents employed in this study to potentiate drug uptake, however, act in a different manner since the intracellular ATP content was not affected (see Results). Nevertheless, the effects on cells of metabolic inhibitors and of the membrane active compounds used in this study show some similarities. Both classes of compounds stimulated colchicine uptake to a maximal rate of about 0.13 pmol/min per 10^6 cells. Moreover, they will stimulate puromycin uptake as well (ref. 3 and Fig. 4). Under conditions of maximal stimulation, the mutant cells with reduced drug permeability attained uptake rates similar to the parental cells; the treated cells were fully viable and the stimulatory effect was reversible (ref. 3 and Ling, V., unpublished). The activation energy of colchicine uptake in cyanide-stimulated and vinblastine-stimulated cells are similar (Fig. 7 and Carlsen, S. A., unpublished). All these observations suggest that the domain of the membrane through which colchicine permeates can exist in a fluid or permeable state (absence of ATP or in the presence of membrane active agents) which is not irreversibly detrimental to the cell, and that under normal conditions the maintenance of a modulated state requires some ATP-linked process.

Some of the agents used in this study, such as vinblastine, vincristine, daunomycin and actinomycin D, which were shown to stimulate drug uptake, are commonly used antineoplastic agents and frequently used in combination chemotherapy. Our results suggest that some of the synergism seen in combination therapy may be due to a "detergent-like" effect in which one drug could potentiate the permeability of another. Indeed Goldman et al. [23] have already proposed the use of vincristine for increasing intracellular levels of methotrexate in high-dose methotrexate-folinic acid reversal protocols.

The observation that colchicine permeates the cell via an unmediated process has at least two fundamental ramifications in our understanding of the mechanism of reduced drug permeability in the colchicine resistant mutants. First, it is perhaps not surprising that a single or limited number of mutations leading to reduced colchicine permeability in Chinese hamster ovary cells also leads to an unexpectedly wide pleiotropic phenotype [1, 2] since, alteration(s) able to modify an unmediated process of transport, might be expected to have relatively broad consequences on

membrane functions. In contrast to this, at least one class of membrane altered mutants, the ouabain resistant cells isolated by Baker et al. [24], have an altered mediated transport system associated with the $(Na^+ + K^+)$-ATPase and these cells display no unexpected pleiotropic features in their phenotype (Baker, R. M., personal communication). Second, we have observed recently that a glycoprotein of approximately 170 000 daltons is exposed at the surface of colchicine resistant mutants and is absent or in greatly decreased amounts in the wild-type cells [25, 26]. Moreover, the amount of this glycoprotein appears to correlate with the degree of reduced permeability in these mutants [27]. These results coupled with the present observations suggest that certain surface glycoproteins are able to modulate the permeability of compounds entering cells via unmediated diffusion. Although the nature of this modulation is not known, it has been proposed that these surface glycoproteins are able to affect membrane fluidity, and thus modulate the uptake of colchicine and a variety of other compounds [26].

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REFERENCES

1 Ling, V. and Thompson, L. H. (1974) J. Cell. Physiol. 83, 103–116
2 Bech-Hansen, N. T., Till, J. E. and Ling, V. (1976) J. Cell. Physiol. 88, 23–31
3 See, Y. P., Carlsen, S. A., Till, J. E. and Ling, V. (1974) Biochim. Biophys. Acta 373, 242–252
4 McBurney, M. W. and Whitmore, G. F. (1974) Cell 2, 173–182
5 Thompson, L. H. and Baker, R. M. (1974) in Methods in Cell Biology (Prescott, D. M., ed.), Vol. VI, pp. 209–281, Academic Press, New York
6 Stanners, C. P., Elicieri, G. and Green, H. (1971) Nat. New Biol. 230, 52–54
7 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
8 Stanley, P. E. and Williams, S. G. (1969) Anal. Biochem. 29, 381–392
9 Stein, W. D. (1967) The Movement of Molecules Across Cell Membranes, pp. 289–295, Academic Press, New York
10 Borisy, G. G. and Taylor, E. W. (1967) J. Cell Biol. 34, 535–548
11 Palmer, C. G., Livengood, D., Warren, A., Simpson, P. G. and Johnson, I. S. (1960) Exp. Cell Res. 20, 198–201
12 Fyfe, M. J. and Goldman, I. D. (1973) J. Biol. Chem. 248, 5067–5073
13 Seeman, P. (1966) Biochem. Pharmac., 15, 1753–1766
14 Seeman, P. (1966) Biochem. Pharmac. 15, 1767–1774
15 Seeman, P. (1972) Pharmacol. Rev. 24, 583–655
16 Seeman, P., Chau-Wong, M. and Moyyen, S. (1973) Nat. New Biol. 241, 22
17 Hubbell, W. L., Metcalfe, J. C., Metcalfe, S. M. and McConnell, H. M. (1970) Biochim. Biophys. Acta 219, 415–427
18 Butler, K. W., Schneider, H. and Smith, I. C. P. (1973) Arch. Biochem. Biophys. 154, 548–554
19 Papahadjopoulos, D., Jacobson, K., Poste, G. and Shepherd, G. (1975) Biochim. Biophys. Acta 394, 504–519
20 de Kruyff, B., de Greef, W. J., van Eyck, R. V. W., Demel, R. A. and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 298, 479–499

21 McElhaney, R. N., de Gier, J. and van der Neut-Kok, E. C. M. (1973) Biochim. Biophys. Acta 298, 500–512
22 Poste, G., Papahadjopoulos, D., Jacobson, K. and Vail, W. J. (1975) Biochim. Biophys. Acta 394, 520–539
23 Goldman, I. D., Gupta, V., White, J. C. and Loftfield, S. (1976) Cancer Res. 36, 276–279
24 Baker, R. M., Brunette, D. M., Mankovitz, R., Thompson, L. H., Whitmore, G. F., Siminovitch, L. and Till, J. E. (1974) Cell 1, 9–21
25 Juliano, R., Ling, V. and Graves, J. (1976) J. Supramol. Struct. 4, 521–526
26 Ling, V. (1975) Can. J. Genet. Cytol. 17, 503–515
27 Juliano, R. and Ling, V. (1976) Biochim. Biophys. Acta, in the press