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INCREASED DRUG PERMEABILITY IN CHINESE HAMSTER OVARY CELLS IN THE PRESENCE OF CYANIDE

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SUMMARY

In this report we investigated whether the modulation of drug permeability in Chinese hamster ovary (CHO) cells was an energy-dependent process. We observed that (1) in the absence of glucose, metabolic inhibitors such as cyanide, azide, and dinitrophenol stimulated the uptake of [^{3}H]colchicine and other drugs; (2) cyanide-induced stimulation of drug uptake could be prevented by the presence of metabolizable sugars such as glucose and ribose; (3) cyanide-treated cells were fully viable; (4) on the addition of cyanide and glucose the kinetics of drug permeability changes were very rapid. These data are consistent with the hypothesis that an energy-dependent membrane barrier against the uptake of a variety of drugs was operative in CHO cells.

The nature of this energy-dependent membrane barrier was examined in colchicine-resistant mutants (CH^RC4 and CH^RC5 cells) previously characterized as membrane mutants with greatly reduced drug permeability (Ling and Thompson, (1974) J. Cell Physiol. 83, 103–116). The mutants were more refractile to the cyanide-induced stimulation of drug permeability but more sensitive to the glucose prevention of cyanide-induction. In the presence of cyanide, the uptake rate of [^{3}H]colchicine by CH^RC4 cells increased by about 100-fold and approached a rate similar to that of wild-type cells. These results suggest that the colchicine-resistant mutants may be altered in their energy-dependent modulation of drug permeability.

INTRODUCTION

The regulation of membrane permeability is an essential property of mammalian cells. Changes in membrane permeability would be expected to reflect alterations in surface membrane structure which could cause modifications in a variety of other cell functions. Recent studies in our laboratory on colchicine-resistant (CH^R) mutants of Chinese hamster ovary (CHO) cells in culture have provided support for this view.

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We have isolated from independent subclones of CHO cells a class of colchicine-resistant mutants which, because their resistance correlates strongly with reduced drug permeability, appear to be membrane-altered mutants [1]. One characteristic feature of these mutants is their pleiotropic cross-resistance to a variety of structurally and functionally unrelated drugs such as actinomycin D, daunomycin, puromycin, vinblastine, erythromycin and cytochalasin B (Ling and Thompson [1], N. T. Bech-Hansen, personal communication). Thus these mutants apparently possess membrane alterations which result in reduced permeability to a wide spectrum of compounds.

A modulation of drug permeability dependent on metabolic energy has been postulated for mammalian cells by various investigators [2, 3] who observed an increased level of drugs in mouse L1210 leukemia cells or Ehrlich ascites tumor cells incubated in the presence of metabolic inhibitors. At present, the mechanism of this energy-dependent process is ill-understood, nor is it known whether such a process is generally operative in all mammalian cells. It was of interest therefore to discover whether an energy-dependent process is associated with the regulation of membrane permeability to colchicine and other drugs in our CHO cell lines.

In this report, we describe experiments which indicate that the inhibition of energy production greatly stimulated the permeability to colchicine in parental and drug-resistant cells; further, their uptake of colchicine in the presence of metabolic inhibitors approached similar rates. This suggests an energy-dependent modulation of a functional membrane barrier against the uptake of colchicine and other drugs in CHO cells and also raises the possibility that this modulation is altered in the CH^R cell mutants.

MATERIALS AND METHODS

Drugs and Chemicals. Colchicine, D(+) xylose, D(−) ribose, β-D(−) fructose, D(+) galactose (substantially glucose free), α-L(−) fucose, 2-deoxyglucose and sodium azide were obtained from Sigma Chemical Company. 2,4-Dinitrophenol was obtained from Calbiochem and D(+) glucose from British Drug Houses. Sugars were assayed for contaminating glucose by an assay employing glucose oxidase obtained in kit form from Sigma Chemical Company (Sigma Kit No. 510). [^{3}H]Colchicine (5 Ci/ mmole) 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.

Cells. The cell lines CH^RC4 and CH^RC5 used in this investigation were colchicine-resistant mutants of Chinese Hamster Ovary cells isolated by Ling and Thompson [1]. The parent of these mutants (AUX B1) was an auxotrophic mutant requiring glycine, adenosine and thymidine in the culture medium for growth [4]. Procedures used for maintaining mutant 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); the medium plus antibiotics and serum is designated complete α-minimum essential medium.

Uptake of radioactive colchicine. Cells were harvested by centrifugation, washed once with phosphate buffered saline and resuspended in the same buffer to a concentration of about $3 \cdot 10^6$ cells/ml. Aliquots of this cell suspension were added to tubes containing [^{3}H]colchicine and test substances in phosphate buffered saline and incubated at 37 °C in a water bath shaker. The amounts of radioactive drug taken up during cell incubations were determined as follows: at appropriate times 1-ml aliquots were removed from each tube, added to one ml phosphate buffered saline containing 20 μM colchicine, immediately centrifuged at maximal speed for 2 min in a table-top centrifuge (Clinical model, International Equipment Co.), and the resulting supernatant removed by aspiration. The cells were washed three more times in phosphate buffered saline containing 20 μM colchicine by centrifugation and resuspension. All washing procedures were performed at room temperature. The final cell pellet was resuspended in 0.5 ml of water and added to a scintillation vial. The centrifuge tube was rinsed with an additional 0.5 ml of water which was added to the same vial and 10 ml of a Triton X-100: Toluene scintillation fluid [7] was then added. The amount of radioactivity in each vial was determined by a liquid scintillation counter (Nuclear Chicago). All samples were corrected for background by subtraction of a zero time sample which contained about 0.05 pmoles labelled colchicine/10^6 cells.

RESULTS

Effect of cyanide on colchicine uptake

Our initial experiments were designed to determine whether the metabolic inhibitor potassium cyanide (KCN) affected colchicine permeability in CHO cells, and whether the parental line (AUX B1 cells) and a colchicine-resistant cell line (CH^{R}C4) differed in their response to the inhibitor. As can be seen in Fig. 1, in agreement with our previous observations [1] the rate of [^{3}H] colchicine uptake by AUX B1 cells was significantly higher than by CH^{R}C4 ($13 \cdot 10^{-3}$ versus less than $0.8 \cdot 10^{-3}$ p mole/min/10^6 cells respectively). However, in the presence of 2 mM KCN, the rates of colchicine uptake in both lines were greatly increased. AUX B1 cells took up

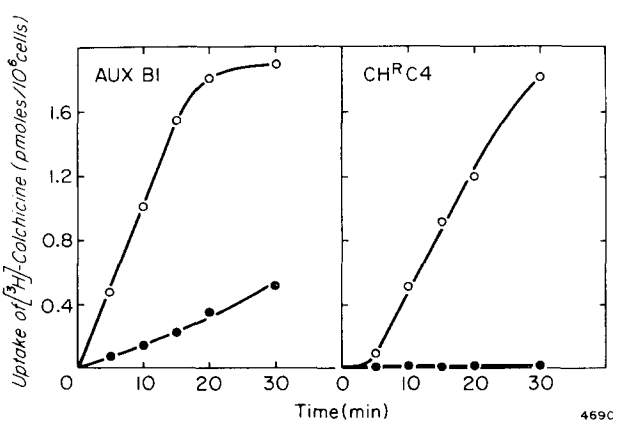

Fig. 1. Effect of KCN on colchicine uptake. Colchicine uptake was measured as described in Materials and Methods using 1 μM [^{3}H] colchicine adjusted to a specific activity of 1 Ci/mmole with unlabelled colchicine; in the presence (○—○) and absence (●—●) of 2 mM KCN.

TABLE I

EFFECT OF METABOLIC INHIBITORS ON COLCHICINE UPTAKE

The amount of [^{3}H] colchicine taken up in the first 15 min of incubation was measured as described in Materials and Methods.

Inhibitor (concentration)	Uptake of [^{3}H]colchicine (cpm · 10^{-2} per 10^6 cells)		
	AUX B1 cells	CH^{R}C4 cells	CH^{R}C5 cells
None	1.6	≤0.2	≤0.2
Cyanide (2 mM)	12	7.5	6.2
Azide (10 mM)	6.5	1.5	2.5
Dinitrophenol (1 mM)	11	3.8	8.6

colchicine at a rate of 105 · 10^{-3} pmole per min per 10^6 cells while CH^{R}C4 cells behaving somewhat differently reached a rate of 93 · 10^{-3} pmole per min per 10^6 cells after an initial lag. This lag could be eliminated by a 5 min preincubation of CH^{R}C4 cells in cyanide prior to the addition of labelled colchicine. It is significant that the uptake of colchicine by AUX B1 cells and CH^{R}C4 cells in the presence of cyanide approached similar rates while in the absence of cyanide they differed by more than 10-fold.

Other metabolic inhibitors also stimulated [^{3}H]colchicine uptake in mutant and wild-type CHO cells. As can be seen in Table I, azide and dinitrophenol were also able to increase drug permeability. These inhibitors had similar effects on another highly colchicine-resistant cell line CH^{R}C5, selected independently from CH^{R}C4 cells

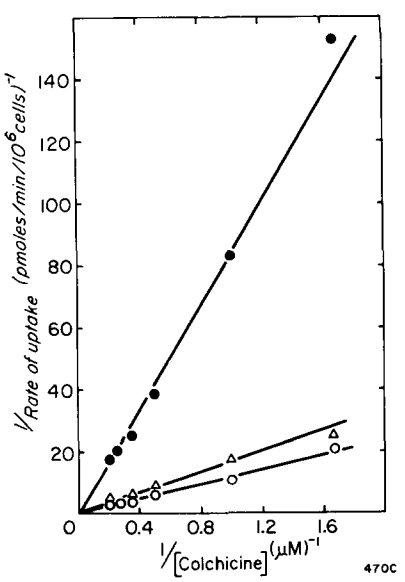

Fig. 2. Concentration dependence of colchicine uptake. Rates were measured from the linear portion of the uptake curves as shown in Fig. 1, for AUX B1 cells (circles) and CH^{R}C4 cells (triangles) in the presence (open symbols) and absence (closed symbols) of 2 mM KCN.

(Ling and Thompson [1]). It should be noted that in this experiment no attempt was made to maximize the rate of colchicine uptake; the amount of [^{3}H] colchicine taken up in the first 15 min of incubation and in the presence of one concentration of inhibitor was measured.

To investigate whether the nature of colchicine uptake stimulated in the presence of cyanide was different from that in the absence of the inhibitor, we measured the rate of [^{3}H] colchicine uptake as a function of colchicine concentration. Lineweaver-Burk plots of the rates of colchicine uptake by AUX B1 and CH^{R}C4 cells in the presence or absence of cyanide are shown in Fig. 2. The curve for CH^{R}C4 cells in the absence of KCN is not shown since the drug uptake rate was too low to be accurately measured. It is seen that all the curves pass through the origin and only their slopes were changed in the presence of cyanide. This finding suggests that increasing concentrations of colchicine do not result in saturation kinetics for the uptake of this drug in our cells and is compatible with the hypothesis that both in the presence and absence of KCN, colchicine enters the cells by the same mechanism with kinetics apparently consistent with passive diffusion. It is possible, of course, that saturation kinetics might occur at higher drug concentrations since in this particular experiment relatively low concentrations of colchicine were assayed (up to $5 \cdot 10^{-6}$ M). However, similar results were obtained in another experiment in which colchicine concentrations up to $1 \cdot 10^{-4}$M were tested in the absence of cyanide.

The effect of different concentrations of cyanide on the uptake of colchicine was determined. As seen in Fig. 3, the amount taken up in AUX B1 and CH^{R}C4 cells increased rapidly with KCN concentrations up to 0.5 mM after which the rate remained relatively constant. In AUX B1 cells, the rate of uptake decreased gradually

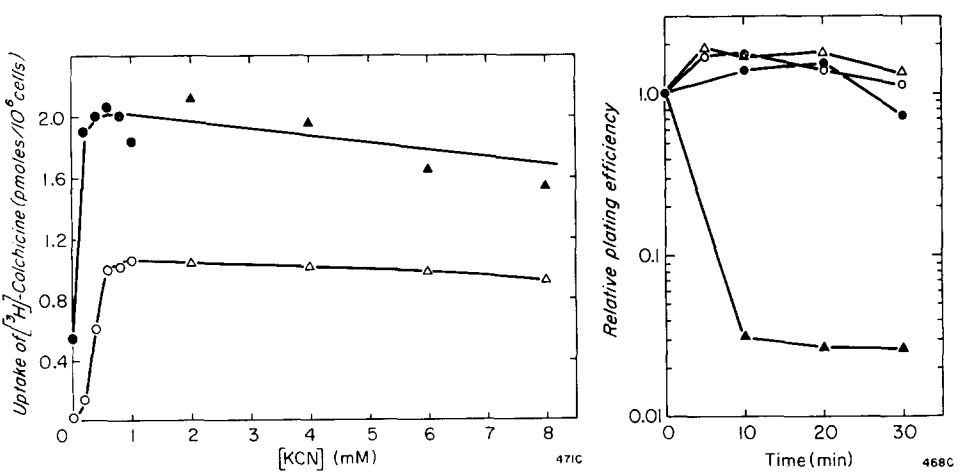

Fig. 3. Effect of KCN concentration on the stimulation of colchicine uptake. The amount of colchicine taken up in the first 15 min of incubation was measured, as described in Materials and Methods, for varying concentrations of KCN for AUX B1 (closed symbols) and CH^{R}C4 (open symbols) (circles and triangles refer to two independent experiments).

Fig. 4. Effect of incubation in KCN on cell viability. Cell line CH^{R}C4 was incubated for varying times at 37 °C in phosphate-buffered saline containing no (●), 1 mM (○), 2 mM (△) or 4 mM (▲) KCN; cells were collected by centrifugation at various times and then plated in complete α-minimal essential medium.

with increasing KCN concentration above 1mM, possibly as a result of cytotoxic effects of KCN. In this case, the maximal uptake of colchicine in CH^RC4 cells appeared to be substantially less than in AUX B1 cells. This is explained by the fact that in this experiment the rate of colchicine uptake was measured from the amount of colchicine taken up in the first 15 min of incubation where as seen in Fig. 1, CH^RC4 cell line has a lag period.

Viability of cyanide-treated cells

While the above results were consistent with the hypothesis that the uptake of colchicine into CHO cells was modulated by an energy-dependent process, it was possible that the amount of KCN employed in our experiments might have been cytotoxic to the cells resulting in a non-specific increase in membrane permeability. For this reason, the viability of the cyanide-treated cells was investigated. The results are presented in Fig. 4, which shows the relative colony-forming ability (relative plating efficiency) of cells treated with different concentrations of KCN under the normal drug-uptake conditions for different lengths of time. It can be seen that CH^RC4 cells treated with 2 mM KCN or lower for 30 min showed no loss in their ability to form colonies. Similar results were obtained for AUX B1 cells. Thus, it appears that even at concentrations of KCN (0.5 mM) which stimulated maximally the rate of colchicine uptake (see Fig. 3), no loss in cell viability was observed. This provides evidence against the possibility that the cyanide-induced permeability was a non-specific toxic effect.

Cyanide effect on the uptake of other drugs

We had found previously [1] that our colchicine-resistant mutants were characterized by a concomitant cross-resistance and reduced permeability to other drugs, such as puromycin, having structures and intracellular targets different from colchicine. On the rationale that the uptake of these drugs might also be affected by

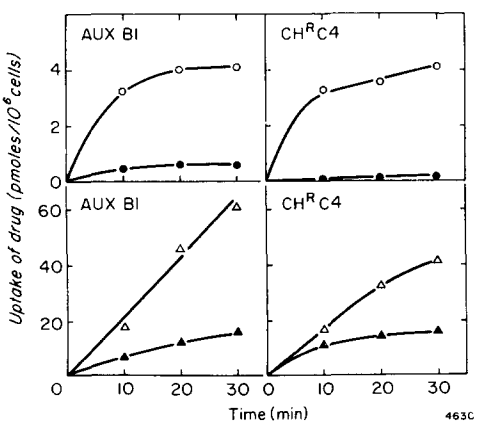

Fig. 5. Effect of KCN on actinomycin D and puromycin uptake. Uptake was measured as described for colchicine in Materials and Methods, except that cells were washed in ice-cold phosphate buffered saline. Uptake was measured using 1 μM [^{3}H] puromycin (circles) or 1 μM [^{3}H] actinomycin D (triangles) in the absence (closed symbols) and presence (open symbols) of 2 mM KCN.

cyanide, we investigated the effects of cyanide on the uptake of actinomycin D and puromycin. Fig. 5 shows the uptake of tritiated puromycin and actinomycin D by AUX B1 and CH^{R}C4 cells, in the presence and absence of 2 mM KCN. As expected, in the absence of cyanide, CH^{R}C4 cells took up puromycin less rapidly than AUX B1 cells. In the presence of KCN, however, both cell lines took up puromycin more rapidly with similar rates. The actinomycin D uptake by parent and mutant cells was also stimulated by cyanide; in this case the stimulated uptake rate of CH^{R}C4 cells was somewhat less than that of AUX B1 cells. These observations demonstrate that KCN induces both AUX B1 and CH^{R}C4 cells to a state of similar permeability to actinomycin D and puromycin in addition to colchicine (Fig. 1). The fact that KCN was able to increase cell permeability to a variety of apparently unrelated drugs suggests that the effect of cyanide was not limited to increasing permeability specifically to a single drug but that by inhibiting energy production in the cell, it was probably altering some more general property of the cell membrane.

Because of this apparent lack of specificity of increased drug permeability in the presence of cyanide, it was important to investigate whether or not the presence of metabolic inhibitors resulted in a non-specific "leakiness" of the cell membranes. Thus we performed experiments to measure the uptake of thiourea and erythritol (thought to enter the cell by passive diffusion), and α-aminoisobutyric acid (thought to enter the cell by facilitated diffusion), into our cells in the presence and absence of cyanide. We observed that the initial uptake rates of these compounds were similar for AUX B1 and CH^{R}C4 cells; further, these rates were not affected by the presence of cyanide up to 2mM. These results are consistent with the concept that in the presence of metabolic inhibitors, the cell membranes do not become generally leaky but rather, the membranes become more permeable to a specific class of unrelated drugs by some mechanism(s) which is not presently understood.

Effect of glucose and other sugars on colchicine uptake

Since the above experiments designed to measure the induction by KCN of an increased colchicine uptake were performed in glucose-free phosphate buffered saline, and since mammalian cells are able to utilize glucose via glycolysis as a source of metabolic energy, we investigated whether the addition of glucose affected the KCN-induced stimulation of drug uptake in our cells. As seen in Fig. 6, in both AUX B1 and CH^{R}C4 cells, the KCN-stimulated [^{3}H] colchicine uptake was little affected in the presence of glucose at concentrations less than 0.1 mM; however, at higher sugar concentrations the uptake rate rapidly decreased until a basal level was approached at glucose concentrations of 5mM or greater. These observations support the hypothesis that our cells utilize glucose to produce metabolic energy in the presence of KCN and consequently prevent the previously observed KCN-induced stimulation of drug permeability. It can be observed also in Fig. 6 that the KCN-induced permeability in CH^{R}C4 cells was more sensitive to glucose than AUX B1; the glucose concentrations at which the rate of [^{3}H] colchicine uptake was half of maximum were 0.3 and 0.7 mM, respectively.

Because it was possible that the above observed effect of glucose on the KCN-induced permeability was mediated via a sugar effect not related to metabolic energy, the ability of different sugars to prevent the KCN-induced drug permeability in AUX B1 and CH^{R}C4 cells was also examined. As seen in Fig. 7, ribose at 15 mM was

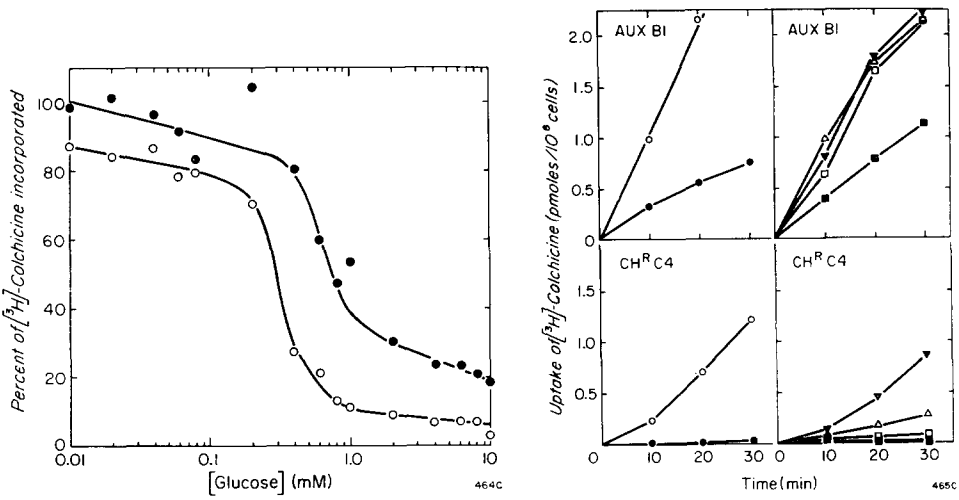

Fig. 6. Effectiveness of different concentrations of glucose in preventing the cyanide-stimulated colchicine uptake. Uptake was performed as described in Fig. 1 in the presence of 2 μM [^{3}H] colchicine, 2 mM KCN and different concentrations of glucose. The amount of [^{3}H] colchicine taken up in the first 15 min of incubation was measured and all values are expressed as the percent of [^{3}H] colchicine taken up by each line in the absence of glucose. ● — ●, AUX B1 cells; ○ — ○, CH^{R}C4 cells.

Fig. 7. The ability of various sugars in preventing the KCN-induced stimulation of colchicine uptake. Uptake was measured as in Fig. 1 in the presence of 2 mM KCN with the following additions: ○ — ○, no sugar; ● — ●, glucose; △ — △, galactose, □ — □, xylose; ▼ — ▼, fucose; ■ — ■, ribose. All sugars were present at 15 mM except in the case of AUX B1 cells where galactose and fucose were used at 7.5 mm.

effective in preventing the cyanide-induction of permeability in both AUX B1 and CH^{R}C4 cells while xylose was more effective in CH^{R}C4 cells but had little effect on AUX B1 cells. Galactose also appeared to have a small effect on CH^{R}C4 cells but this could be due to the presence of some contaminating glucose (0.84 mole % as measured by the glucose oxidase assay (Materials and Methods)). The amount of contaminating glucose measured by the same assay in ribose (0.01 mole %) and xylose (0.45 mole %) probably could not account for their observed effect of preventing cyanide induction. In addition to the sugars already mentioned, fructose and 2-deoxyglucose, up to 15 mM were also tested but these did not reverse the cyanide effect. These results suggest that the prevention of KCN-induced drug permeability by glucose was not a non-specific sugar effect since only a few sugars other than glucose e.g. ribose and possibly xylose, were able to do this. It is perhaps significant that in some mammalian cells at least, ribose and xylose could be metabolized for energy via the pentose–phosphate pathway [8, 9]. Thus, these findings are consistent with the concept that glucose and other sugars prevent KCN from effecting increased drug permeability by supplying the cell with a source of metabolic energy not inhibited by cyanide.

Rate of permeability changes

The above results suggest that the maintenance of a membrane permeability barrier against the uptake of drugs in CHO cells was associated with energy metab-

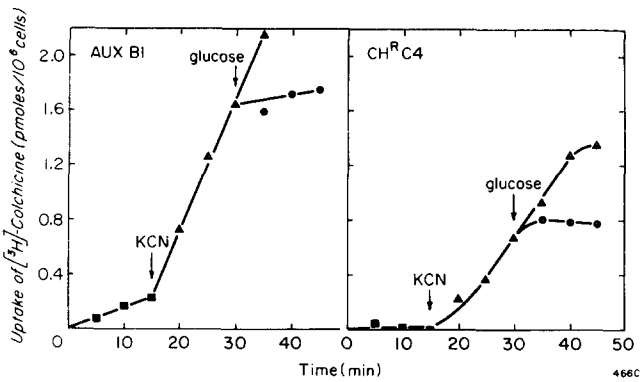

Fig. 8. Reversibility and kinetics of KCN-induced stimulation of colchicine uptake. Uptake of colchicine was measured as in Fig. 1 in the absence of KCN (■—■). At 15 min KCN was added to a final concentration of 2 mM (▲—▲). At 30 min an aliquot was taken and glucose added to a final concentration of 15 mM (●—●).

olism, possibly with the metabolic energy level (e.g. ATP level) in the cell. The ATP level in mammalian cells has been observed to undergo rapid changes under different conditions of cyanide inhibition and in the presence of glucose [11, 12]. To test whether the drug permeability changes in our system also underwent rapid changes, cells were incubated in [^{3}H] colchicine and their rates of drug uptake measured after treatment with KCN and glucose as described in Fig. 8. It was observed that in both AUX B1 and CH^{R}C4 cells there was a rapid increase in the uptake of colchicine on the addition of 2 mM KCN. This increased permeability was just as quickly reversed on the addition of 15 mM glucose. The fact that the KCN-induced permeability could be reversed with glucose further demonstrates that KCN was not irreversibly damaging the cells, in support of the evidence presented in Fig. 4 that KCN-treated cells were fully viable. The relative rapidness of the permeability changes observed in Fig. 8 suggests that a close coupling exists between the modulation of membrane permeability to drugs and the metabolic energy level in our cells.

DISCUSSION

We have presented evidence in this report that metabolic energy is required for the maintenance of a functional membrane barrier against a variety of drugs in CHO cells. For example, we have demonstrated (1) that in the absence of glucose, a number of metabolic inhibitors (cyanide, azide, and dinitrophenol) stimulated the uptake of colchicine and a class of apparently unrelated drugs into independent lines of CHO cells (Table I, Fig. 5); yet, they have relatively little effect on the uptake of compounds such as thiourea, aminoisobutyric acid and erythritol: (2) that the KCN-induced stimulation of drug permeability could be prevented and reversed by the presence of sugars such as glucose (Fig. 6 and Fig. 8) and ribose (Fig. 7) which could be metabolized by the cell for energy: (3) that the kinetics of permeability changes were very rapid (Fig. 8) and could correlate with rapid changes in ATP levels. This is discussed in more detail below.

We have also observed differences in the response of the parental line and a colchicine-resistant mutant to cyanide (Fig. 1 and Fig. 3) and sugars (Fig 6 and Fig. 7)

with respect to their drug permeability. These observations, coupled with the fact that in the presence of cyanide both the mutant and parent cell acquired relatively similar drug permeability, raise the possibility that the mutation in the colchicine-resistant cell involves an alteration in the energy-requiring drug-permeability barrier of the cell. A more detailed analysis of other independently selected lines of colchicine-resistant cells will be required to determine whether the altered response to metabolic inhibitors and to sugars as observed in this investigation for CH^RC4 cells is generally associated with colchicine resistance. For this purpose it will be important also to examine revertants of the drug-resistant cells for the reversion of these responses.

Other investigators [2, 3] have also observed an energy-dependent barrier against the uptake of drugs into mammalian cells. Goldman [3] reported the stimulation of methotrexate uptake into murine leukemia cells incubated in the presence of sodium azide. In his system, the presence of azide did not appear to alter the initial rate of drug uptake although the steady-state level of methotrexate in the cell was stimulated by about two-fold. Danø [2] also observed an increase in the steady-state level of daunomycin in Ehrlich ascites cells in the presence of metabolic inhibitors. To account for this increase in the steady-state levels of drugs, these investigators suggested that an energy-dependent carrier-mediated drug-efflux system for the maintenance of a drug permeability barrier may be present in mammalian cells.

While our data do not contradict this hypothesis, we have made observations in our system which are different from the above studies [2, 3] and which suggest that the energy-dependent permeability barrier may be more complex than just an efflux system. First, unlike Goldman's and Danø's observations, we have observed in our system great increases in the initial rates of drug uptake into CHO cells in the presence of metabolic inhibitors. Second, we could not observe saturating kinetics for the uptake of colchicine with concentrations up to $1 \cdot 10^{-4}$M. This suggests that colchicine is transported across the cell membranes either by passive diffusion or by some putative drug carrier system which must be present in our cells in relatively high concentrations. Third, the energy-dependent permeability barrier has specificity for a class of apparently unrelated drugs. This conclusion is arrived at by the observation that the uptake of colchicine, puromycin and actinomycin D is stimulated by cyanide, while the uptake of thiourea, erythritol and α-aminoisobutyric acid is not.

While the mechanism for maintaining the energy-dependent membrane barrier is not understood, the colchicine-resistant cells possess features which should prove useful for the further investigation of this mechanism. For example, as can be seen in Fig. 1, the CH^R line is highly sensitive to the cyanide-induced stimulation of drug permeability; permeability increased by 100-fold in CH^RC4 cells compared with 8-fold for the parental line. The cyanide-treated cells are fully viable (Fig. 4) for at least 30 min and the cyanide-induced permeability could be readily reversed with glucose (Fig. 8). Finally, since the CH^R cells appear to have membrane alterations that are related to the process(es) regulating membrane permeability, various altered forms of this process(es) may be available for study with different CH^R mutants.

The rapidity of the drug permeability changes observed in Fig. 8 suggests that the modulation of this permeability involves a dynamic membrane controlled by a rapidly-modulated factor(s) associated with energy metabolism. While it is recognized that the number of possible molecules involved here may be extensive, several observations are consistent with the concept that ATP could be such a factor. First, the

ATP level in mammalian cells can undergo rapid changes. For example we have observed that the level of ATP in both parental and CH^R mutant cells (as measured by the firefly luciferase assay [10]) decreases with a half-life of about 1 min in the presence of 2 mM cyanide (unpublished observation). These results support those of Plagemann and Erbe [11] with Novikoff hepatoma cells, and Coe and Greenhouse [12] with Ehrlich ascites cells, who have made the additional observation that the low ATP level of cells incubated in the presence of cyanide quickly returned to normal on the addition of glucose. Second, in certain cases at least, the apparent fluidity of mammalian cell membrane appears to be associated with the level of ATP within the cell. For example, it has been suggested that the ability of cells to agglutinate in the presence of concanavalin A is a reflection of the fluid state of the cell membrane [13–15]. Vlodavsky and coworkers [14] have shown with transformed hamster embryo fibroblasts that cells gained agglutinability (increased membrane fluidity) after treatment with the metabolic inhibitor 2, 4-dinitrophenol which greatly reduced the intracellular ATP content. Furthermore, these DNP-treated cells lost their agglutinability after treatment with glucose which restored their normally high intracellular ATP content. Since these observations parallel so exactly the observations of drug permeability changes in our cells as presented in Fig. 8, it seems entirely possible that alterations in the cell membranes resulting in increased cell agglutinability also results in increased drug permeability in our system.

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REFERENCES

1 Ling, V. and Thompson, L. H. (1974) J. Cell Physiol. 83, 103–116
2 Danø, K. (1973) Biochim. Biophys. Acta. 323, 466–483
3 Goldman, I. D. (1969) J. Biol. Chem. 244, 3779–3785
4 McBurney, M. 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 Keast, D. and Bartholomaeus, W. N. (1973) J. Immunol. Methods 2, 261–267
8 Hiatt, H. H. (1957) J. Biol. Chem. 224, 851–859
9 Chang, R. S. (1957) Proc. Soc. Exp. Biol. and Med. 96, 818–820
10 Stanley, P. E. and Williams, S. G. (1969) Anal. Biochem. 29, 381–392
11 Plagemann, P. G. W. and Erbe, J. (1973) J. Cell Physiol. 81, 101–112
12 Coe, E. L. and Greenhouse, W. V. V. (1973) Biochim. Biophys. Acta. 329, 171–182
13 Nicholson, G. L. (1972) Nat. New Biol. 239, 193–197
14 Vlodavsky, I., Inbar, M. and Sachs, L. (1973) Proc. Natl. Acad. Sci. U.S. 70, 1780–1784
15 Guérin, C., Zachowski, A., Prigent, B., Paraf, A., Dunia, I., Diawara, M. and Benedetti, E. L. (1974) Proc. Natl. Acad. Sci. U.S. 71, 114–117