Nucleoside Transport in Mammalian Cells. Inhibition by Colchicine†

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ABSTRACT: Colchicine inhibited the transport of nucleosides in several mammalian cell lines. The action of colchicine on transport was reversible, although incompletely, upon removal of the colchicine from the medium. Inhibition of adenosine, guanosine, uridine, and thymidine transport by colchicine appeared to be competitive. The inhibition constants for colchicine were approximately 6×10^{-5} M, and were essentially the same for all of the different transport systems. Inhibition of transport appeared specific for nucleosides since colchicine did not prevent the uptake of 2-deoxyglucose or α-aminoisobutyric acid. The effect of colchicine on nucleoside transport appears unrelated to an action on microtubules. Lumicolchicine, an analog of colchicine which does not disrupt microtubules, or bind to microtubule protein, inhibited thymidine and uridine transport 50% at 2×10^{-5} and 4×10^{-5} M, respectively. However, podophyllotoxin and several colchicine analogs which do interact with microtubule protein, were effective inhibitors of thymidine transport. Colchicine had no effect on the synthesis of DNA or RNA, nor did it inhibit the *in vitro* phosphorylation of thymidine or uridine.

Although mammalian cells do not require exogenous nucleosides for growth, cells will take up nucleosides and utilize them for the synthesis of nucleic acids if present in the extracellular environment. Investigations with density-inhibited cells have demonstrated that several nucleoside transport systems are sensitive to environmental factors such as pH (Ceccarini and Eagle, 1971) and serum addition (Cunningham and Pardee, 1969; Weber and Rubin, 1971). For example, when cells are released from density-dependent growth inhibition upon addition of serum, one of the earliest observable events is an increase in nucleoside transport.

The transport of nucleosides in mammalian cells is thought to occur by a facilitated diffusion process, which involves specific permeation reactions (Jacquez, 1962; Kessel and Shurin, 1968; Plagemann, 1971). However, little is known of the mechanisms involved. It is also not known whether the nucleoside transport and phosphorylation reactions are functionally or structurally coupled as suggested for sugar transport in yeast (Van Steveninck, 1969) and in several bacterial systems (Kaback, 1970).

A number of chemical agents have been found to inhibit nucleoside transport (Scholtissek, 1968b; Plagemann and Roth, 1969; Plagemann, 1970; Schuster and Hare, 1970). For example, thymidine transport in hamster embryo cells is inhibited by inorganic and organic mercurials (Schuster and Hare, 1970), while nucleoside transport in Novikoff rat hepatoma cells (Plagemann and Roth, 1969; Plagemann, 1970) and chick embryo cells (Scholtissek, 1968b) is prevented by persantine. The results presented in this communication demonstrate that the antimitotic agent, colchicine, inhibits specifically the transport of nucleosides in several mammalian cell lines. The site for inhibition of nucleoside transport by colchicine does not appear to be the subunits of microtubules, but rather, a membrane component(s) of the separate nucleoside transport systems.

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Methods

Cell Culture. HeLa cells (human cervical carcinoma) were grown in monolayer culture in Roux or 6-oz. prescription bottles at 37° on Joklik-modified medium (Schwarz Biochemical Research Inc.) supplemented with 7% fetal calf serum (Grand Island Biological Co.). KB (human oral carcinoma), WI-38 (human fetal lung), and L929 mouse fibroblasts were grown under similar conditions. CCF (normal human skin fibroblasts), V79 (Chinese hamster fibroblasts), and EHB (Syrian hamster fibroblasts) were grown in monolayer culture on McCoy's 5a medium (modified) (Grand Island Biological Co.) supplemented with 20% fetal calf serum.

Cells were kindly tested for the presence of mycoplasma by Dr. L. Hayflick, Department of Medical Microbiology, Stanford University; no contamination occurred during this investigation. Cell number was determined with a Coulter counter. Cell viability was determined utilizing erythrosin B which is excluded by viable cells.

Uptake Experiments. ASSAY OF RADIOACTIVITY IN CELLS. Incorporation of radioactivity into trichloroacetic acid soluble and insoluble material was assayed according to the procedure of Klevecz and Stubblefield (1967) with modification. Approximately 16 hr prior to use, cells were trypsinized with 0.25% trypsin (Grand Island Biological Co.) and transferred to sterile glass scintillation vials at a cell density of 10^5 cells/vial to ensure logarithmic growth. The concentration dependence for the inhibition of nucleoside uptake by colchicine was determined in the following manner. Growth medium was aspirated and 1 ml of fresh assay medium containing desired concentrations of drug was added to each vial. Vials were then incubated for 1 hr at 37°. Serum was omitted from the assay medium for two reasons. (1) Colchicine and lumicolchicine were found to bind to a serum factor(s) (unpublished data) and (2) both thymidine and uridine uptake were reduced in the presence of serum, possibly due to the presence of nucleosides in serum (Forsdyke, 1971). After initial incubation with drug, assay medium was aspirated and fresh assay medium containing labeled nucleoside and appropriate concentrations of drug was added for an additional 10-min

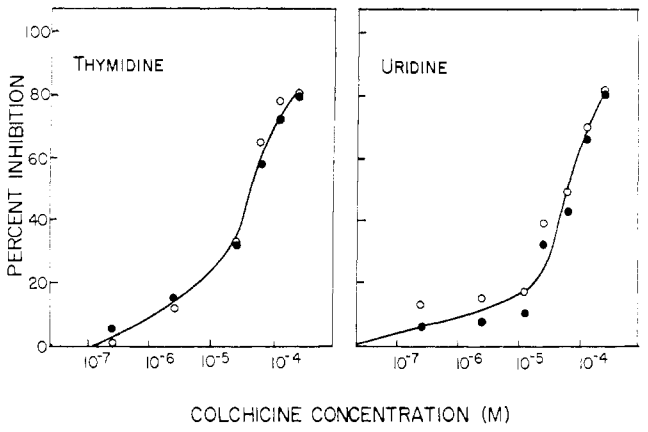

FIGURE 1: Concentration dependence of inhibition of thymidine and uridine uptake by colchicine. Cells were incubated with increasing concentrations of colchicine for 1 hr prior to 10-min incubation with 1.5×10^{-7} M [^{3}H]thymidine (1 μCi/ml) or 3.7×10^{-6} M [^{14}C]-uridine (0.2 μCi/ml); trichloroacetic acid soluble (●); trichloroactic acid insoluble (○).

incubation. The cell layer was then rapidly washed three times with balanced salt solution (BSS) at 0°. Vials were incubated with 1 ml of 10% trichloroacetic acid at 0–4° for 2 hr. The trichloroacetic acid soluble material was removed and an aliquot of this material was prepared for determination of radioactivity by incubating with NCS Solubilizer (Amersham/Searle) for 2 hr at 37°. The remaining cell layer (trichloroacetic acid insoluble; DNA or RNA) was washed twice with ice-cold 10% trichloroacetic acid and incubated with NCS Solubilizer as above. Scintillation fluid containing 5 g of Omnifluor (New England Nuclear) per l. toluene was added and the radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer, Model 3310.

The effects of colchicine and related analogs on the initial rates of uptake of nucleosides into trichloroacetic acid soluble and insoluble material was determined by simultaneous addition of labeled nucleoside and drug to vials at zero time. Samples were taken at 5-min intervals and assayed for radioactivity as described above. Corrections were made for zero time uptake, *i.e.*, radioactivity which was immediately associated with the cells following the addition and removal manipulations of medium containing labeled nucleoside. The data represent the average of duplicate samples, which differed by less than 10% in all cases. Counting efficiency for ^{3}H was 39%; for ^{14}C, 85%.

NUCLEOSIDE KINASE ASSAYS. Thymidine and uridine kinase activities were assayed according to the method of Kitt and Dubbs (1965) with slight modification. Approximately 3×10^7 cells were scraped from a Roux bottle and washed three times with BSS at 0°. Cells were suspended in 1 ml of 0.15 M KCl, 3 mM mercaptoethanol, and 10 mM Tris-HCl (pH 8.5), and sonicated with a Bronwill Biosonik III Sonifier for 20 sec. The sonicate was centrifuged at 10,000*g* for 10 min, and the particulate material was discarded. Assay mixtures for thymidine kinase activity contained 9 mM ATP, 9 mM $MgCl_2$, 7.5 mM 3-phosphoglycerate, 50 mM Tris-HCl (pH 8.5), 150 μl of cell supernatant (110–120 μg of protein), and 1.88×10^{-7} M [^{3}H]thymidine (5 μCi/assay), in the presence or absence of 3.1×10^{-4} M colchicine; final volume 405 μl. Incubations were carried out at 37°. Aliquots (100 μl) were removed at 0, 5, and 10 min and placed in a boiling water bath for 2 min. Particulate material was removed by centrifugation at 1500*g* for 5 min. Aliquots of the supernatants (50 μl) were applied to 2.4-cm^2 DEAE-81 filter paper disks. Thymidine (or uridine) was removed from the disks by five 100-ml washes with 10 mM sodium phosphate buffer (pH 6.8) at 0°. Only the phosphorylated nucleoside derivatives remained on the positively charged DEAE-81 filter paper disks. Filter paper disks were placed in scintillation vials with 0.5 ml of NCS solubilizer and incubated for 2 hr at 37° prior to addition of scintillation fluid. Uridine kinase activity was measured with 1×10^{-5} M [^{14}C]uridine (0.25 μCi/assay). Protein was determined by the method of Lowry *et al.* (1951).

RADIOACTIVITY AND CHEMICALS. [^{3}H]Thymidine (6.7 Ci/mmole), [^{14}C]uridine (54.1 Ci/mole), [^{3}H]guanosine (11.7 Ci/mmole), [^{3}H]adenosine (15.5 Ci/mmole) [^{3}H]2-deoxyglucose (6.8 Ci/mmole), [^{3}H]α-aminoisobutyric acid (128 Ci/mole), and [^{32}P]phosphate (200 mCi/mg) were purchased from New England Nuclear Corp.

Colchicine was a gift from Eli Lilly Co. Podophyllotoxin was obtained from Aldrich Chemical Co. Colcemid was purchased from CIBA. Milligram quantities of β- and γ-lumicolchicine (referred to as lumicolchicine) were prepared by modification of a method described previously (Wilson and Friedkin, 1966). Solutions of colchicine at 5.3×10^{-5} M in absolute ethanol were irradiated in hand-blown quartz 100-ml round-bottom flasks with a Blak-Ray long-wave ultraviolet lamp (UVL-22; Ultraviolet Products Inc., San Gabriel, Calif.). Solutions were irradiated for 90 min (half-time = 12 min at 25°) and the completion of the reaction was ascertained by examination of ultraviolet spectra (β- and γ-lumicolchicine: λ_{max} 267 nm in 100% ethanol, ϵ 23,600). Actinomycin D (Cosmegen) was purchased from Merck Sharp & Dohme. Cytosine arabinoside was obtained from Sigma.

Results

Inhibition of Nucleoside Uptake by Colchicine. Colchicine inhibited the uptake of adenosine, guanosine, thymidine, and uridine into HeLa cells in a concentration-dependent manner. The concentration dependence for inhibition of thymidine and uridine uptake into trichloroacetic acid soluble and insoluble material by colchicine is shown in Figure 1. Fifty per cent inhibition of both thymidine and uridine uptake occurred with approximately 5×10^{-5} M colchicine. Identical results were obtained with adenosine and guanosine (data not shown). The data in Figure 1 were obtained with cells incubated with colchicine for 1 hr prior to addition of labeled nucleoside. Almost identical results were obtained when colchicine and nucleoside were added simultaneously to cells, except that at concentrations of colchicine below 2.5×10^{-5} M, the effect of colchicine on uptake was time dependent. For example, thymidine and uridine uptake were unaffected by 2.5×10^{-6} M colchicine when nucleoside and drug were added simultaneously to cells, while uptake was inhibited 15–20% when cells were incubated with 2.5×10^{-6} M colchicine for 1 hr prior to addition of thymidine or uridine.

The incorporation of thymidine and uridine into trichloroacetic acid insoluble material was not preferentially inhibited by colchicine, relative to incorporation into trichloroacetic acid soluble material (Figure 2). Several investigations have demonstrated that an effect on nucleoside uptake of a drug such as persantine or an environmental factor such as pH or serum addition, results in a parallel degree of inhibition of incorporation into trichloroacetic acid soluble and

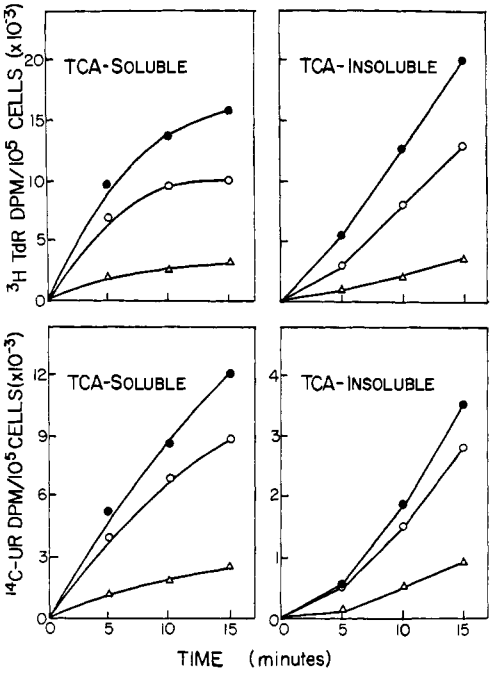

FIGURE 2: Time course for thymidine and uridine incorporation into trichloroacetic acid soluble and trichloroacetic acid insoluble material in the presence and absence of colchicine. Cells were incubated with 1.5×10^{-7} M [^{3}H]thymidine (upper) or 3.7×10^{-6} M [^{14}C]uridine (lower) in the presence or absence of colchicine; control (●); 2.5×10^{-5} M colchicine (○); 2.5×10^{-4} M colchicine (Δ).

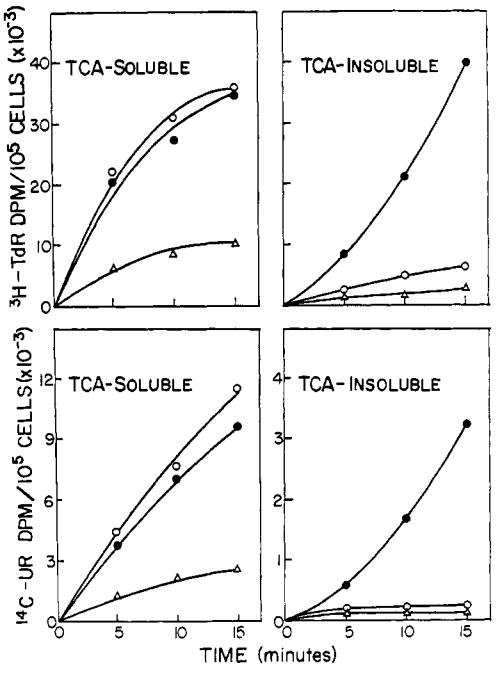

FIGURE 3: Effect of colchicine, cytosine arabinoside, and actinomycin D on thymidine and uridine uptake and incorporation into trichloroacetic acid soluble and insoluble material in HeLa cells. Thymidine uptake (upper) was assayed in cells incubated for 5, 10, and 15 min at 37° with 1.5×10^{-7} M [^{3}H]thymidine (1 μCi/ml) (●); [^{3}H]thymidine and cytosine arabinoside (10 μg/ml) (○); and [^{3}H]-thymidine, cytosine arabinoside (10 μg/ml), and 2.5×10^{-4} M colchicine (Δ). Uridine uptake (lower) was assayed in cells incubated during a similar time period with 3.7×10^{-6} M [^{14}C]uridine (0.2 μCi/ml) (●); [^{14}C]uridine and actinomycin D (5 μg/ml) (○); and [^{14}C]uridine, actinomycin D (5 μg/ml), and 2.5×10^{-4} M colchicine (Δ).

insoluble material, while inhibition of DNA or RNA synthesis results in a preferential inhibition of nucleoside incorporation into trichloroacetic acid insoluble material (Plagemann and Roth, 1969; Cunningham and Pardee, 1969). Additional support for the conclusion that colchicine was not inhibiting nucleic acid synthesis was obtained utilizing known inhibitors of DNA and RNA synthesis. The addition of cytosine arabinoside (Chou and Fischer, 1962) or actinomycin D (Reich, 1963) to cells did not result in a decrease in the size of trichloroacetic acid soluble pools of thymidine and uridine nucleotides relative to control samples, while nucleoside incorporation into trichloroacetic acid insoluble material was inhibited (Figure 3). Addition of colchicine with cytosine arabinoside or actinomycin D caused a decrease in the trichloroacetic acid soluble pools of nucleotides, without further significant inhibition of nucleotide incorporation into trichloroacetic acid insoluble material. These results support the conclusion that colchicine does not affect nucleic acid synthesis, but rather inhibits some aspect of nucleoside uptake in HeLa cells. This conclusion was further supported by the results of experiments with [^{32}P]phosphate. The incorporation of labeled phosphate into DNA or RNA was unaffected by colchicine, under conditions where nucleoside uptake was maximally affected.

Effect of Colchicine on Thymidine and Uridine Kinase Activity in Vitro. In vitro phosphorylation of either [^{3}H]thymidine or [^{14}C]uridine was unaffected by colchicine (Table I). The marked lability of thymidine kinase activity at low substrate concentrations (Kitt and Dubbs, 1965) may be responsible for the nonlinearity of the phosphorylation of thymidine. However, the phosphorylation of thymidine was enzymatic as evidenced by the lack of phosphorylation in the absence of cell supernatant.

Inhibition of Nucleoside Uptake by Colchicine Analogs and Podophyllotoxin. A possible role of microtubules in the inhibition of nucleoside uptake was considered unlikely based on experiments with lumicolchicine, an analog of colchicine which is inactive as an antimitotic agent and which does not interact with microtubule protein (Wilson and Friedkin, 1967). Fifty per cent inhibition of thymidine and uridine uptake occurred with 2×10^{-5} and 4×10^{-5} M lumicolchicine, respectively (Table II). Furthermore, inhibition of nucleoside uptake by colchicine occurred at 0° to a similar extent as at 37°. This finding also indicates that microtubules are not involved in nucleoside uptake, since the interaction of colchicine with microtubule protein is temperature dependent,

TABLE I: Effect of Colchicine on Phosphorylation of Thymidine and Uridine *in Vitro*.

Nucleoside	nmole of Nucleoside Phosphorylated/μg of Sonicate Protein 5 min	10 min
[^{3}H]Thymidine		
Control	2.4×10^{-6}	3.1×10^{-6}
Colchicine (3.1×10^{-4} M)	2.3×10^{-6}	2.9×10^{-6}
[^{14}C]Uridine		
Control	1.3×10^{-3}	2.5×10^{-3}
Colchicine (3.1×10^{-4} M)	1.1×10^{-3}	2.3×10^{-3}

TABLE II: Concentrations of Colchicine Analogs and Podophyllotoxin Which Inhibit Thymidine and Uridine Uptake by 50%.

Drug	Drug Concentration (mole/l.) [^{3}H]Thymidine	[^{14}C]Uridine
Colchicine	4×10^{-5}	6×10^{-5}
Lumicolchicine	2×10^{-5}	4×10^{-5}
Colcemid	4×10^{-5}	*a*
Isocolchicine	2.5×10^{-4}	*a*
Podophyllotoxin	6×10^{-6}	*a*

[a] Not determined. Cells were incubated for 1 hr with drug prior to 10 min of incubation with 1.5×10^{-7} M [^{3}H]thymidine (1 μCi/ml) or 3.7×10^{-6} M [^{14}C]uridine (0.2 μCi/ml).

TABLE IV: Apparent Kinetic Constants for the Incorporation of Adenosine, Guanosine, Thymidine, and Uridine into HeLa Cells. Determination of K_i for Colchicine.

Nucleoside	K_m (M)	V_{max} (nmoles/10 min per 10^5 Cells $\times 10^3$)	K_i (M)
Adenosine	2×10^{-5}	67	6.6×10^{-5}
Guanosine	3.6×10^{-6}	670	5.0×10^{-5}
Thymidine			
37°	2.4×10^{-7}	8.3	6.1×10^{-5}
0°	1×10^{-6}	1.3	9.4×10^{-5}
Uridine	8.3×10^{-6}	200	4.5×10^{-5}

being markedly reduced at 0° (Wilson and Friedkin, 1967). However, podophyllotoxin (Table II) and several analogs of colchicine (*e.g.*, colcemid and isocolchicine) which do exhibit antimitotic activity, inhibited nucleoside uptake. Colcemid was as effective as colchicine, while podophyllotoxin was more effective, inhibiting thymidine uptake 50% at 6×10^{-6} M.

General Characteristics of Nucleoside Uptake Inhibition by Colchicine. The effect of colchicine on transport was specific for nucleosides. The uptake of a nonmetabolizable sugar, 2-deoxyglucose (Smith and Gorski, 1968), and a nonmetabolizable amino acid, α-aminoisobutyric acid (Kuchler and Kuchler, 1965), were unaffected by colchicine (data not shown). These findings suggest that the effect of colchicine on nucleoside transport is not the result of an effect on all cellular transport processes.

The inhibition of thymidine and uridine uptake by colchicine was reversible, although incompletely, upon removal of the colchicine from the medium. Cells growing in scintillation vials were incubated for 1 hr at 37° in the presence or absence of 2.5×10^{-4} M colchicine, washed three times with BSS at 37°, and incubated with labeled nucleoside for 30 min. Thymidine and uridine uptake were still inhibited 20–30% 30 min after colchicine was removed from the assay buffer. However, in experiments with HeLa cells growing in suspension culture, the inhibition of thymidine and uridine uptake was immediately and completely reversible. Increasing the number of BSS washings of the cells assayed in the scintillation vials had no effect on the retention of nucleoside uptake inhibition. The lack of complete reversibility with monolayer cells is most likely not due to a cytotoxic effect of colchicine, since viability studies revealed no significant differences in terms of cell number or viability in HeLa cells incubated in the presence or absence of 2.5×10^{-4} M colchicine for 2 hr.

TABLE III: Effect of 2.5×10^{-4} M Colchicine on Thymidine and Uridine Uptake in Several Cell Lines.

Cell Line	% Inhibn of [^{3}H]TdR Uptake	% Inhibn of [^{14}C]UR Uptake
Human		
CCF	96	92
WI-38	92	94
HeLa	80	81
KB	78	79
Hamster		
Chinese (V79)	31	43
Syrian (EHB)	24	33
Mouse		
L929	41	*a*

[a] Not determined. Cells were incubated in the presence or absence of colchicine for 1 hr at 37°. [^{3}H]Thymidine (1.5×10^{-7} M, 1 μCi/ml) or [^{14}C]uridine (3.7×10^{-6} M, 0.2 μCi/ml) was added and incubation was continued for an additional 10 min. Uptake was determined as described in Methods.

Effect of Colchicine on Thymidine and Uridine Uptake in Several Cell Types. The inhibition of thymidine and uridine uptake by colchicine was not restricted to HeLa cells, but was also observed in a number of other human and rodent cell lines (Table III). Interestingly, all of the human lines were at least ten times more sensitive than the rodent cell lines. Furthermore, thymidine transport was unaffected by colchicine in *Escherichia coli*.

Kinetics of Nucleoside Uptake Inhibition by Colchicine. Inhibition of thymidine, uridine, adenosine, and guanosine uptake by colchicine appeared to be competitive (Figure 4). Inhibition of thymidine uptake by colchicine at 0° was also competitive although total uptake in control samples was reduced 80–90%, relative to samples at 37° (data not shown). The Michaelis constants (K_m), values of maximal velocity (V_{max}), and the inhibition constants (K_i), derived from Lineweaver–Burk plots are shown in Table IV. Although the K_m values are analogous to the Michaelis constants of Michaelis–Menten kinetics, expressing some type of *affinity* of substrate for the transport system, in the formal sense, they merely express the substrate concentration at which uptake is one-half the maximal velocity (Stein, 1967). The values obtained for K_m and V_{max} agree with published values for Novikoff rat hepatoma cells (Plagemann, 1971) and chick embryo cells (Scholtissek, 1968b).

Adenosine, guanosine, thymidine, and uridine utilize separate transport systems in HeLa cells (unpublished data), Novikoff rat hepatoma cells (Plagemann, 1971), and in chick embryo cells (Steck *et al.*, 1969). It is therefore of considerable interest that the inhibition constants (K_i) for colchicine are not significantly different. These findings suggested the

possibility that the separate transport systems shared a common factor(s), with which colchicine interacted to inhibit nucleoside uptake. However, this cannot be the case since the inhibition of [^{14}C]uridine uptake by colchicine could not be reversed by the addition of thymidine (10^{-5} M) nor could the inhibition of [^{3}H]thymidine uptake by colchicine be reversed by addition of uridine (10^{-5} M).

Discussion

Colchicine has been reported to inhibit DNA and RNA synthesis in several systems (Hell and Cox, 1963; Ilan and Quastel, 1966; Williams, 1968; Fitzgerald and Brehaut, 1970). However, we have not found any preferential inhibition of nucleoside incorporation into trichloroacetic acid insoluble material (DNA or RNA) relative to trichloroacetic acid soluble material by colchicine. Experiments with actinomycin D and cytosine arabinoside demonstrated that colchicine did not affect nucleic acid synthesis, but inhibited some aspect of nucleoside uptake. Inhibition of uptake appeared to be specific for nucleosides, since colchicine did not prevent the uptake of either 2-deoxyglucose or α-aminoisobutyric acid.

The effect of several colchicine analogs and podophyllotoxin on nucleoside transport is interesting in view of the interactions of these agents with the protein subunits of microtubules (Wilson, 1970). Since microtubule protein does not interact with lumicolchicine, and the binding of colchicine to microtubule protein is markedly reduced at 0°, the demonstration that lumicolchicine inhibited thymidine and uridine transport (Table II) and that colchicine prevented thymidine transport at 0° (Table IV), suggests that microtubules are not associated with the effect of podophyllotoxin and the colchicine analogs on transport. However, the different site(s) for transport inhibition and microtubule protein interaction do exhibit a similar spectrum of reactivity toward these agents, although the concentration range of colchicine for transport inhibition is at least one to two orders of magnitude higher than that necessary for disruption of microtubules (*cf.* Schindler, 1965). Podophyllotoxin, which appears to interact at the same binding site on microtubule protein as does colchicine (Wilson, 1970; Bryan, 1972), was more effective than colchicine in preventing transport, while colcemid, an analog of colchicine which is equipotent with colchicine as an antimitotic agent (Schindler, 1965) was as effective as colchicine as an inhibitor of transport (Table II). Trimethylcolchicinic acid (3.5×10^{-5} M) an analog of colchicine which, like lumicolchicine does not interact with microtubule protein (unpublished data), unlike lumicolchicine, did not inhibit transport. The binding of colchicine to microtubule protein appears to involve a hydrophobic interaction (Wilson, 1970). Similar hydrophobic areas on proteins involved in transport (Lieb and Stein, 1971) may be the target sites for the colchicine analogs and podophyllotoxin.

The results of kinetic studies (Figure 4, Table IV) indicate that inhibition of nucleoside transport by colchicine is competitive and that the inhibition constants are essentially the same for all four nucleosides. The findings that the four nucleoside transport systems are separate, and that the addition of either thymidine or uridine could not reverse the inhibitory effect of colchicine on [^{14}C]uridine or [^{3}H]thymidine transport, respectively, suggest that colchicine does not affect a factor(s) common to all four transport systems, but rather, interacts with similar factors which are associated with the separate transport systems.

There are several possible sites for the inhibition of nucleoside transport by colchicine. One possibility is that colchicine acts directly at the initial nucleoside binding site of the separate transport systems. This appears unlikely because of the apparent specificity of the separate adenosine, guanosine, thymidine, and uridine transport systems. However, it is possible that the transport binding sites for the separate systems may have sufficient structural similarities such that they have a similar affinity for colchicine. The phosphorylation of thymidine or uridine was unaffected by colchicine *in vitro;* however, this does not preclude the possibility of an effect on a postulated transport-associated phosphorylation reaction in the intact cell membrane (Scholtissek, 1968a; Schuster and Hare, 1971). This is also considered unlikely due to the substrate specificity of the various nucleoside kinases (Scholtissek, 1968a). A third possibility is that colchicine interacts with a membrane component in proximity to, or in association with, the transport carrier, which results in steric hinderance of transport.

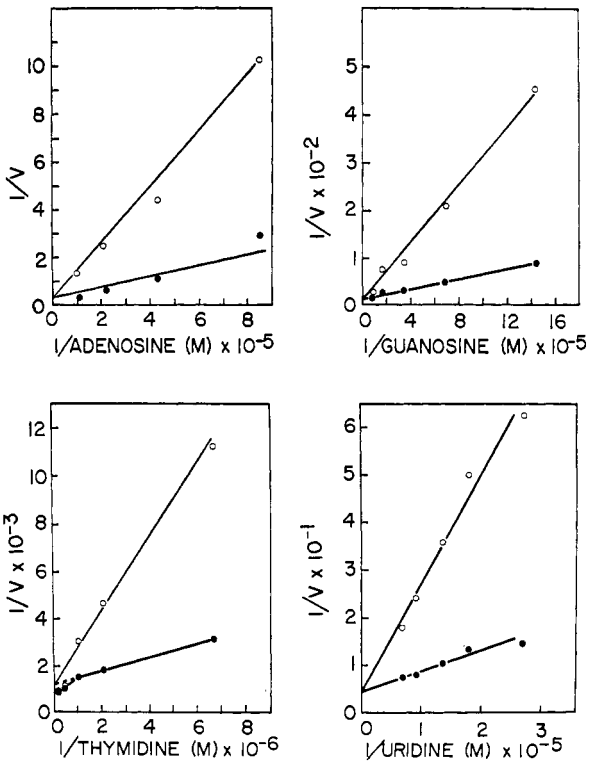

FIGURE 4: Lineweaver–Burk plots for the inhibition of nucleoside uptake by colchicine in HeLa cells. Cells were incubated for 10 min at 37° with several concentrations of nucleosides in the presence (○) or absence (●) of 2.5×10^{-4} M colchicine. *V*, nmoles/10 min per 10^5 cells.

Peterson *et al.* (1967) have suggested that nucleoside transport in *Escherichia coli* may be mediated by at least two components: (1) an initial nucleoside binding component which exhib ts substrate specificity (permease), and (2) a nonspecific carrier element to which the permeases transfer nucleosides, and which is responsible for substrate movement across the cell membrane. It is possible that such a system exists in mammalian cells and that colchicine interacts with the transport carrier rather than with the initial nucleoside binding elements. This site of action of colchicine would satisfy the kinetic data (Figure 4, Table IV), which suggests the presence of similar (or identical) components in the separate transport system.

Finally, an intriguing possibility is that colchicine may

interact at sites on the separate transport carriers which may be essential for transport regulation. Such regulatory sites could be sufficiently similar so that each site would exhibit the same affinity for colchicine. Furthermore, similar regulatory sites for the separate transport systems would provide a mechanism for the coordinated control of nucleoside uptake under different environmental conditions.

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Nucleoside Antibiotics. Asymmetric Incorporation of Glutamic Acid and Acetate into the Maleimide Ring of Showdomycin by *Streptomyces showdoensis*†

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ABSTRACT: Carbon-14 from [1-^{14}C]acetate, [2- and 5-^{14}C]-glutamate, and [5-^{14}C]α-ketoglutarate, but not [1-^{14}C]α-ketoglutarate, resides in the carbonyl carbons of the maleimide ring. Degradation of the maleimide ring of showdomycin showed that all of the ^{14}C from the [1-^{14}C]acetate or [5-^{14}C]-glutamate resides in C-5 of showdomycin while all of the ^{14}C from [2-^{14}C]glutamate resides in C-2 of showdomycin. Radioactivity from [2,3-^{14}C]succinate, [2,3-^{14}C]fumarate, and [2-^{14}C]acetate was found in all four carbons of the maleimide ring of showdomycin. Apparently the Krebs cycle and the malic enzyme in *Streptomyces showdoensis* function in the conversion of fumarate and succinate to showdomycin. Further proof that C-4 of glutamate or α-ketoglutarate forms the carbon–carbon bond with D-ribose was obtained in an experiment with [5-^{14}C,4-^{3}H]glutamate. While ^{14}C is incorporated into the maleimide ring all of the tritium is lost. Therefore, the four-carbon maleimide ring arising from C-2 to C-5 of α-ketoglutarate must be an asymmetrical unit when condensation occurs with D-ribose to form showdomycin.

Recent studies on the biosynthesis of showdomycin by *Streptomyces showdoensis* have shown that C-2, -3, -4, and -5 of α-ketoglutarate serve as the four-carbon precursor for the maleimide ring of this antibiotic (Elstner and Suhadolnik, 1971a). All of the ^{14}C in showdomycin from [1-^{14}C]acetate, [2-^{14}C]glutamate, and [5-^{14}C]glutamate resided in the car-

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