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Defective Transport of Thymidine by Cultured Cells Resistant to 5-Bromodeoxyuridine

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A line of HeLa cells resistant to 5-bromo-2′-deoxyuridine (BUdR) was established by continuous culture in growth medium containing BUdR; during the selection period, BUdR concentrations, initially 15 μM, were gradually increased to 100 μM. Cells of a clone (HeLa/B5) established from this line were also resistant to 5-fluoro-2′-deoxyuridine (FUdR), but not to the free base, 5-fluorouracil. Although extracts of HeLa/B5 cells exhibited levels of thymidine kinase activity comparable to those of parental cells, rates of uptake of BUdR, FUdR, and thymidine into intact cells were much reduced. The kinetics of uptake of uridine and adenosine, nucleosides which appear to be transported independently of thymidine in HeLa cells, were similar for HeLa/B5 and the parental line (HeLa/0). Relative to thymidine uptake by HeLa/0 cells, that by HeLa/B5 cells was distinctly less sensitive to nitrobenzylthioinosine (NBMPR), a specific inhibitor of nucleoside transport in various types of animal cells. Despite this difference in NBMPR sensitivity, both cell lines possessed the same number of high affinity NBMPR binding sites per mg cell protein. The altered kinetics of thymidine uptake and the NBMPR insensitivity of that function in HeLa/B5 cells suggest that resistance to BUdR is due to an altered thymidine transport mechanism.

Key words: thymidine transport, nitrobenzylthioinosine, bromodeoxyuridine resistances, HeLa cells, thymidine kinase

It is generally held that the entry of nucleosides into animal cells is mediated by nucleoside-specific transport mechanisms (1–4). This is clearly evident in the case of human erythrocytes which, because of their inability to phosphorylate or cleave either uridine or thymidine, afforded an opportunity to study the transport of these compounds in the absence of cellular metabolism (4). The participation of a classical facilitated diffusion process in the movement of these permeants across the erythrocyte plasma membrane was recognized by rate saturability, competition between permeants, nonconcentrative permeation, and accelerative exchange diffusion (3–5). Under circumstances in which nucleoside permeants are subjected to cellular metabolism, it has not been possible to

Abbreviations: NBMPR – nitrobenzylthioinosine, 6-[(4-nitrobenzyl)-thio]-9-β-D-riboturanosylpurine FU – 5-fluorouracil; BUdR – 5-bromo-2′-deoxyuridine; FUdR – 5-fluoro-2′-deoxyuridine; HEPES – N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; MEM – Minimal Essential Medium

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clearly determine whether transport or the metabolic events involved in the uptake process determine the kinetics of that process. In this report, "uptake" refers to the total quantity of a permeant found to be cell-associated after incubation of those cells with the permeant under defined conditions; the term includes both unaltered permeant and all metabolites thereof.

Kinetic studies (1, 2) have shown the uptake process to have the following characteristics in cultured cells of several types: a) rate saturability, b) competition between permeants, and c) inhibition of uptake by compounds that do not affect nucleoside phosphorylation. These characteristics indicate that a rate-limiting step in nucleoside uptake is mediated; it has been generally assumed that transport is that rate-limiting step and, accordingly, initial rate kinetics have been interpreted in terms of transport.

After brief uptake intervals, nucleosides are found in cells primarily in the form of nucleotide metabolites; however, it is not clear whether transport, or a subsequent metabolic step such as phosphorylation, which might even be coupled to the transport event, is rate-limiting in the uptake of particular nucleosides (6–13). For example, studies of the kinetics of thymidine metabolism and uptake in HeLa cells, using a rapid sampling procedure, demonstrated the approximate equivalence of early rates of uptake and thymidine phosphorylation (6). In Novikoff hepatoma cells (1) and HeLa cells (6–9), several nucleoside uptake mechanisms are distinguishable in terms of permeant specificity. It is not known whether this specificity is imparted to the nucleoside uptake process at the level of transport or at that of nucleoside metabolism. Recent studies of thymidine transport in ATP-depleted Novikoff hepatoma cells (10) have identified a low affinity mechanism which appears to resemble the nucleoside transport mechanism of human erythrocytes (3–5). Plagemann et al. (10) have suggested that in Novikoff cells thymidine uptake may proceed a) by a process in which the rate-limiting step in thymidine uptake is phosphorylation rather than transport, or b) by way of 2 systems, a facilitated diffusion system with low substrate specificity, together with a second system which may involve substrate phosphorylation.

In the present work, HeLa cells were selected for BUdR resistance in an attempt to obtain variants with defects in the membrane transport mechanism for thymidine. A number of examples of BUdR resistance in cultured cells have been attributed to decreased thymidine kinase activity (14–17); alteration in thymidine transport has also been suggested as a basis for BUdR resistance (18, 19).

In this report, we compare the properties of a BUdR-resistant clone (HeLa/B5) with those of the parental cell line (HeLa/0) in respect to kinetics of nucleoside uptake, thymidine kinase activity, and sensitivty to nitrobenzylthioinosine (NBMPR), a potent and specific inhibitor of nucleoside transport (20). Previous studies with human erythrocytes demonstrated that NBMPR occupancy of high affinity binding sites decreased uridine transport in proportion to the number of sites occupied (21, 22). HeLa cells possess similar high affinity binding sites for NBMPR, occupancy of which results in a general inhibition of nucleoside uptake (6–9, 23). We have recently shown that a) the thymidine kinase activity of HeLa cell extracts was not inhibited by NBMPR at concentrations greatly in excess of those which inhibited thymidine uptake in intact HeLa cells, and b) the relative proportions of thymidine anabolites in HeLa cells were similar in the absence and presence of NBMPR, although in the latter instance, total anabolism of thymidine was drastically reduced (6). These results are consistent with the view that NBMPR inhibits the transport step which initiates the anabolism of thymidine in HeLa cells.

MATERIALS AND METHODS

Chemicals

[5-^{3}H] BUdR was obtained from New England Nuclear Corporation, Boston, Massachusetts; other labeled compounds were purchased from Amersham/Searle, Oakville, Ontario. Dr. S. R. Naik prepared NBMPR by an established method (24) from 6-thioinosine generously provided by Developmental Therapeutics Program, National Cancer Institute, Bethesda, Maryland. [^{35}S] NBMPR was prepared by G. J. Lauzon of this laboratory (23).

Cell Culture

A cell line resistant to BUdR was selected by serial passage of HeLa S3 cells in monolayer culture in medium containing BUdR, initially at 15 μM; as subculturing proceeded, the BUdR concentration was increased progressively to 100 μM. From the resistant line, a clone (HeLa/B5) was established; characterization of BUdR resistance in HeLa/B5 cells will be described elsewhere (25). The HeLa/B5 clone was chosen for transport studies because of an apparent decrease in thymidine uptake activity relative to the parental cells (HeLa/0). HeLa/B5 cells had a slightly slower proliferation rate and were somewhat larger than HeLa/0 cells; values for cell volume, DNA, and protein content were 1.5–1.7 times larger in the resistant cells (25).

HeLa/0 and HeLa/B5 cells were maintained as monolayer cultures in antibiotic-free Eagle's minimal essential medium (MEM) supplemented with 10% calf serum. After 6 to 8 weekly transfers, cultures were restarted from frozen stocks; the latter were consistently free of Mycoplasma (Dr. J. Robertson, Department of Medical Bacteriology, University of Alberta).

Measurements of cell proliferation rates, nucleoside uptake rates, and [^{35}S]NBMPR binding employed replicate monolayer cultures prepared by the following procedure (6). Inocula from stock monolayer cultures were expanded into suspension cultures in spinner flasks which subsequently became inocula for suspension cultures in 2-liter round bottom flasks kept under continuous agitation with a vibrating mixer (Vibro-Mixer, Model E1, Chemapec, Hoboken, New Jersey); the latter cultures were single-cell suspensions. Throughout this procedure, cultures were maintained in logarithmic growth by dilution with growth medium to keep cell concentrations between 0.5 and 1.0 $\times$ 10^5 cells/ml. The medium for suspension cultures (MEM-S) consisted of calcium-free MEM supplemented with 5% calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mM HEPES (pH 7.4) Replicate monolayer cultures (50 or more) were established in 2-oz prescription bottles (Brockway Glass, Brockway, Pennsylvania) with cells from Vibro-Mixer suspension cultures in attachment medium (MEM with 10% calf serum, HEPES, and antibiotics) and were incubated at 37°C in 5% CO_2 and air. Cell culture materials were purchased from Grand Island Biological Company, Calgary, Alberta.

Nucleoside Uptake Assay

Uptake of ^{3}H-nucleosides by replicate monolayers was measured at 20–21°C using a rapid sampling procedure described previously (6); the uptake medium (MEM-T) consisted of bicarbonate-free MEM supplemented with 12 mM NaCl, 20 mM HEPES buffer (pH 7.4, 20°C), and ^{3}H-nucleosides. Uptake assays using 24-h monolayer cultures (approximately 10^6 cells/bottle) were performed in triplicate, and each culture was pro-

cessed individually as follows. After removing growth medium, the culture bottle was placed horizontally with cells uppermost, 4 ml of MEM-T was added, and the assay was initiated by rapid rotation of the bottle 180° about its long axis to immerse cells. Five seconds before the uptake interval ended, MEM-T was removed by suction and, to terminate the assay, the cell sheet was rapidly flooded with 60 ml of ice-cold 0.154 M NaCl solution (saline). Fifteen seconds later, the saline was removed by aspiration and the bottle was drained thoroughly. After digestion of cell sheets in 1.5-ml portions of NCS tissue solubilizer (Amersham/Searle), 10.0 ml of Bray's fluor (26) was added to each bottle and a sample from each was taken for liquid scintillation counting. For determination of protein content (27), replicate cultures were processed as described above, as far as completion of the draining step.

Thymidine Kinase Activity

Thymidine kinase (ATP:thymidine 5′-phosphotransferase, EC 2.7.1.75) was determined in extracts of replicate monolayer cultures, prepared as follows. After 1 wash with cold saline, to each monolayer was added 1.0 ml of extraction buffer (10 mM NaF; 0.01 M KCl; 2 mM dithioerythritol; 25 mM 6-aminocaproic acid; 20 μM thymidine; 0.01 M Tris-HCl, pH 7.4; 10% glycerol), and after successive freezing (dry ice-ethanol) and thawing steps (repeated 4 times, with mixing after thawing), extracts were centrifuged (4 min, 13,000 × g). Supernatant fractions were reserved for determination of protein content (27), and thymidine kinase activity using the method of Lee and Chang (28). In the latter, cell extracts were incubated for timed intervals in a medium containing 30 μM [^{3}H] thymidine and the thymidine phosphates formed were isolated on DEAE cellulose paper (29); the ^{3}H-content of such samples was determined by liquid scintillation counting after combustion in a Packard Model 306 Sample Oxidizer.

Affinity Chromatography and Polyacrylamide Gel Electrophoresis

Thymidine kinase affinity gels were prepared by the methods of Kowal and Markus (30) as modified by Lee and Cheng (28). p-Aminophenylthymidine-3′-phosphate was linked in the presence of 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide to a Sepharose 4B (Pharmacia, Montreal, Quebec) derivative prepared by CNBr activation followed by reaction with 6-aminocaproic acid (31). Suspensions of HeLa cells (10^7 cells/ml) in extraction buffer (see above) were disrupted with 15-sec bursts of 20-kc ultrasound, and after precipitation with streptomycin sulfate, ammonium sulfate fractions were prepared (28). The resulting precipitates were dissolved in buffer (0.01 M Tris-HCl (pH 7.4), 10% glycerol, 2 mM dithioerythritol, 0.5 mM EDTA) and dialyzed overnight at 4°C against 500 ml of the same buffer. Portions (1.0 ml) of such dialysates were applied to thymidine kinase affinity gel columns (7.0 × 0.5 cm) which were eluted with appropriate buffers (28); eluate fractions (2.0 ml) were assayed for thymidine kinase activity. Crude extracts and fractionated, dialyzed extracts were subjected to electrophoresis on 5% polyacrylamide gels (28).

[^{35}S] NBMPR Binding Assay

To quantitate the binding of NBMPR to HeLa cells, a modification of a procedure described previously (23) was employed. Replicate monolayer cultures were exposed to 4-ml portions of medium (MEM-T) containing [^{35}S] NBMPR at various concentrations for analysis; cell sheets were then rinsed with 60 ml of cold saline and dissolved in 1.5-ml portions of NCS tissues solubilizer. After addition of Bray's fluor solution to the latter,

samples were assayed for ^{35}S activity (as in the nucleoside uptake assay) to determine cell-associated [^{35}S] NBMPR. Medium samples (1.0 ml), after addition to 13.5 ml of Bray's solution, were assayed for ^{35}S activity to determine the concentration of free NBMPR.

RESULTS

Cells of the BUdR-resistant line from which the HeLa/B5 clone was isolated possessed levels of thymidine kinase activity comparable to those of HeLa/0 cells; however, thymidine uptake rates were significantly lower in the former than in the latter under comparable conditions (25). These and other related characteristics were found in cells of the HeLa/B5 clone. The experiments of Table I demonstrated that HeLa/B5 cells were resistant to BUdR and FUdR. Cells of the parental line, HeLa/0, ceased proliferation after 48 h of exposure to BUdR (100 μM), or to FUdR (0.01 μM), whereas proliferation of HeLa/B5 cells was unaffected. Both lines were equally sensitive to FU, suggesting that the resistant character of HeLa/B5 cells was due to altered uptake or metabolism of the nucleoside.

That BUdR/FUdR resistance was a consequence of impaired uptake of these agents is indicated by a comparison of the uptake of radioactive BUdR, FUdR, and thymidine by HeLa/0 and HeLa/B5 cells. Representative time courses of uptake are presented in Fig. 1 for thymidine uptake by both cell types, and reciprocal plots of uptake rates and substrate concentrations (thymidine, BUdR, and FUdR) are presented in Fig. 2. Uptake data have been expressed in terms of cellular protein content rather than cell number be-

TABLE I. Effect of FUdR, BUdR, and FU on Proliferation of HeLa/0 and HeLa/B5 Cells*

	HeLa/0		HeLa/B5	
Agent (μM)	Cell number ($\times 10^{-6}$)	Percent	Cell number ($\times 10^{-6}$)	Percent
Expt. I: FUdR				
0	2.27	100	1.05	100
0.01	0.39	17	1.00	95
0.05	0.10	4	1.02	97
0.1	0.07	3	0.78	74
1.0	0.07	3	0.15	14
Expt. II: BUdR				
0	2.83	100	1.16	100
50	0.42	15	0.98	85
100	0.33	12	0.96	83
Expt. III: FU				
0	3.51	100	1.42	100
1	1.76	50	1.16	82
5	0.38	11	0.39	28

*Replicate monolayer cultures were started with 1×10^5 cells in 3.5 ml of MEM-A medium containing 10% calf serum; 4 h later, 0.5 ml of medium without additives (control) or analog-containing medium was added. Growth curves for the 2 cell types in the presence and absence of the analogs were determined from cell numbers measured after particular intervals of growth at 37°C; 3 bottles for each condition (agent and concentration) were withdrawn for determination of cell number. Only those data for cell numbers after 96 h of growth are presented above (25).

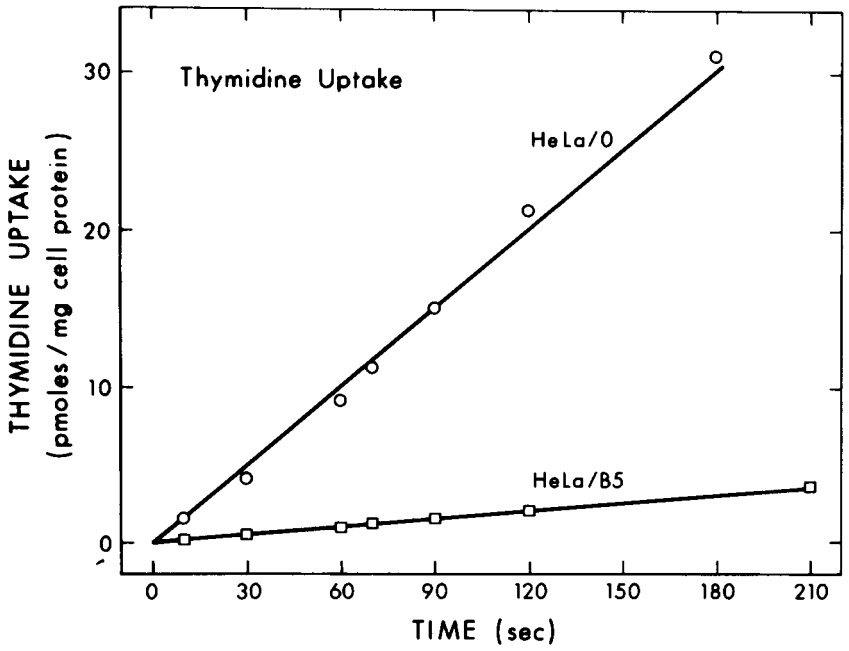

Fig. 1. Time course of thymidine uptake by HeLa/0 and HeLa/B5 cells. Replicate monolayer cultures were exposed at 20°C to MEM-T medium containing 0.05 μM [^{3}H-methyl] thymidine for the intervals indicated. Cellular content of labeled permeant was determined as specified in Materials and Methods.

cause HeLa/B5 cells are larger than HeLa/0 cells (25). It should be noted that uptake of thymidine and thymidine analogs by HeLa/B5 cells is a mediated process, as indicated by the linear reciprocal plots of Fig. 2 and the sensitivity of thymidine uptake to NBMPR (see below).

Because phosphorylation of BUdR and FUdR by thymidine kinase is an essential step in the manifestation of the cytotoxicity of these agents, the properties of the thymidine kinases of HeLa/0 and HeLa/B5 cells were compared. Kinetic studies of the thymidine kinase activity of unfractionated "freeze-thaw" extracts from HeLa/0 and HeLa/B5 cells showed little difference in the apparent kinetic constants for this enzyme activity; with thymidine as the substrate, these values were obtained: K_m, 8–10 μM; V_{max}, 400–450 pmoles thymidine phosphorylated/min/mg protein. When either crude or partially purified extracts from HeLa/B5 and HeLa/0 cells were chromatographed on the thymidine kinase affinity gel, elution profiles for thymidine kinase activity were essentially the same for extracts from either cell type (Fig. 3). Electrophoresis of crude cell extracts on 5% polyacrylamide gels revealed similar profiles of thymidine kinase activity for both cell types (Fig. 4); evidently the slower component (approximately 90% of the total activity) was the cytosol enzyme, while the faster component was of mitochondrial origin (16, 17, 28). These experiments indicated that a) the thymidine kinase content of the variant and parental cells was similar, and b) the kinetic characteristics and the physicochemical properties that determine chromatographic and electrophoretic mobility of the thymidine kinases were similar in both cell types. Thus, the resistance of HeLa/B5 cells

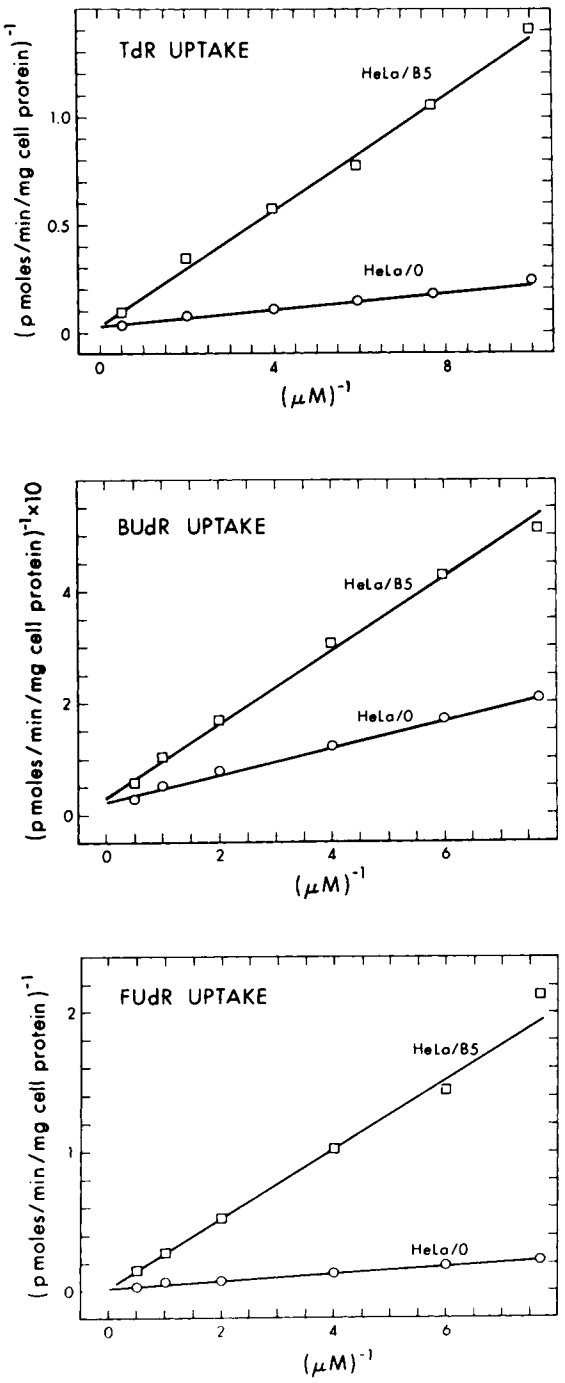

Fig. 2. Kinetics of uptake of thymidine, BUdR, and FUdR by HeLa/0 and HeLa/B5 cells. Replicate monolayer cultures were exposed at 20°C for 60 sec to MEM-T medium containing the indicated concentrations of ^{3}H-labeled permeant and the cellular content of permeant was then determined as given in Materials and Methods. Redrawn from Lynch et al. (25).

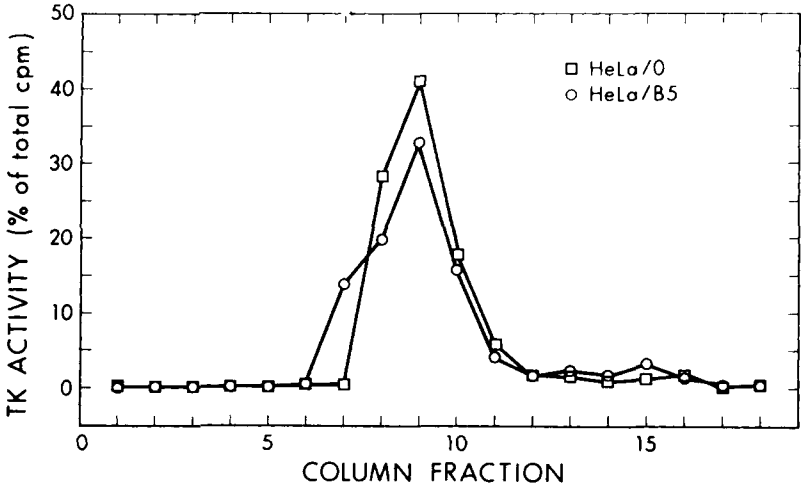

Fig. 3. Elution profiles from thymidine kinase affinity chromatography of extracts from HeLa/0 and HeLa/B5 cells. "Freeze-thaw" extracts, prepared from cells proliferating exponentially in suspension culture, were fractionated with ammonium sulfate and dialyzed overnight (28). Samples (1 ml, 4–6 mg protein) of the fractionated extracts were applied to 0.5 × 7 cm columns of thymidine 3'-phosphate-linked sepharose gel which were eluted with Tris-HCl buffer (pH 7.5) containing 10% glycerol, 5 mM dithioerythritol, and graded concentrations of thymidine. Samples (20 μl) from each eluate fraction (2.0 ml) were assayed for thymidine kinase activity as in Fig. 4.

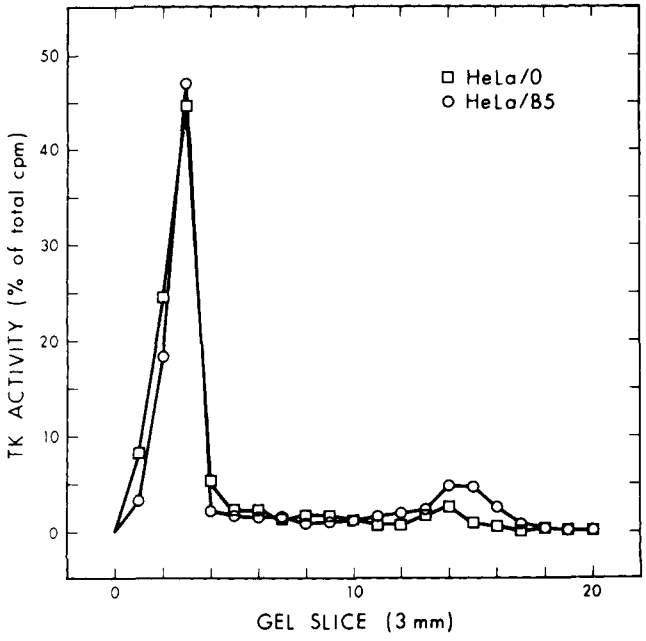

Fig. 4. Electropherogram of thymidine kinase activity from HeLa/0 and HeLa/B5 cells. Samples (50 μl) of "freeze-thaw" extracts, prepared from cells proliferating exponentially in suspension culture, were subjected to electrophoresis on 5% polyacrylamide gels. Gel slices (3 mm) were assayed for thymidine kinase activity by incubation for 1 h at 37°C with 150 μl of the [^{3}H-methyl] thymidine-containing assay mixture as described in Materials and Methods, and the reaction product was isolated on DEAE-cellulose paper for assay of ^{3}H activity.

to the thymidine analogs did not appear to be attributable to changes in thymidine kinase activity. The possibility that the substrate specificity of the thymidine kinase in the variant cells might be altered was not investigated.

We considered that BUdR/FUdR resistance might result from defects in the operation of a membrane transport mechanism; to test this possibility, several aspects of nucleoside uptake were compared in both cell types. Uptake data have been expressed in terms of cellular protein content because HeLa/0 and HeLa/B5 cells differ in size; apparent kinetic constants for uptake of thymidine, thymidine analogs, and for adenosine and uridine are listed in Table II. The 2 cell types did not differ in respect to kinetic constants for uptake of adenosine and of uridine; however, K_m values for uptake of thymidine, BUdR, and FUdR by HeLa/B5 cells were significantly higher than those found with HeLa/0 cells, whereas V_{max} values were essentially identical for uptake of all 3 permeants.

TABLE II. Apparent Kinetic "Constants" for Nucleoside Uptake by HeLa/0 and HeLa/B5 Cells*

Permeant	HeLa/0		HeLa/B5	
	K_m	V_{max}	K_m	V_{max}
BUdR	1.0	43	2.8	42
Thymidine	0.6	32	4.0	30
FUdR	1.3	47	11.3	45
Uridine	6.0	117	4.5	110
Adenosine	3.6	285	2.0	333

*Nucleoside uptake rates were derived from the amounts of ^{3}H-nucleoside taken up by replicate monolayer cultures during 60 sec of incubation at 20°C; rates were measured at various nucleoside concentrations and constants were estimated from reciprocal plots of rates and concentrations (25): K_m, μM, V_{max}, pmoles/min/mg protein.

HeLa/B5 cells were less responsive than HeLa/0 cells to inhibition of thymidine uptake by NBMPR, a potent inhibitor of nucleoside transport. The concentration of NBMPR that reduced thymidine uptake to 50% of control values (IC_{50}) in HeLa/B5 cells was 4 μM; this IC_{50} value is about 800 times higher than that reported previously (6) for HeLa/0 cells (NBMPR inhibition of thymidine uptake, IC_{50} = 0.05 μM). Because NBMPR inhibits transport of thymidine without effect on thymidine metabolism in HeLa cells, the reduced sensitivity of HeLa/B5 cells to NBMPR suggests altered function of the thymidine transporter. The IC_{50} values for NBMPR inhibition of uptake of adenosine and guanosine by HeLa/B5 and HeLa/0 cells were approximately 0.05 μM, indicating that transport of these nucleosides was unaffected in the variant cells (25).

To determine if changes in the number of NBMPR binding sites, or in properties of the latter, could explain reduced NBMPR sensitivity of the thymidine uptake mechanism in HeLa/B5 cells, binding of [^{35}S]NBMPR to monolayer cultures was examined (Fig. 5). Mass law analysis of these binding data by the method of Scatchard (32) indicated that HeLa/0 and HeLa/B5 cells possess similar numbers of NBMPR binding sites (per mg cellular protein) and that the affinity of these sites for NBMPR is similar in both cell types. The mass law plot indicates that binding at low NBMPR concentrations was due to a single type of high affinity binding site; assuming that 1 molecule of NBMPR was bound to each such site, HeLa/B5 and HeLa/0 cells bound 1.24 and 1.25 pmoles NBMPR/mg total

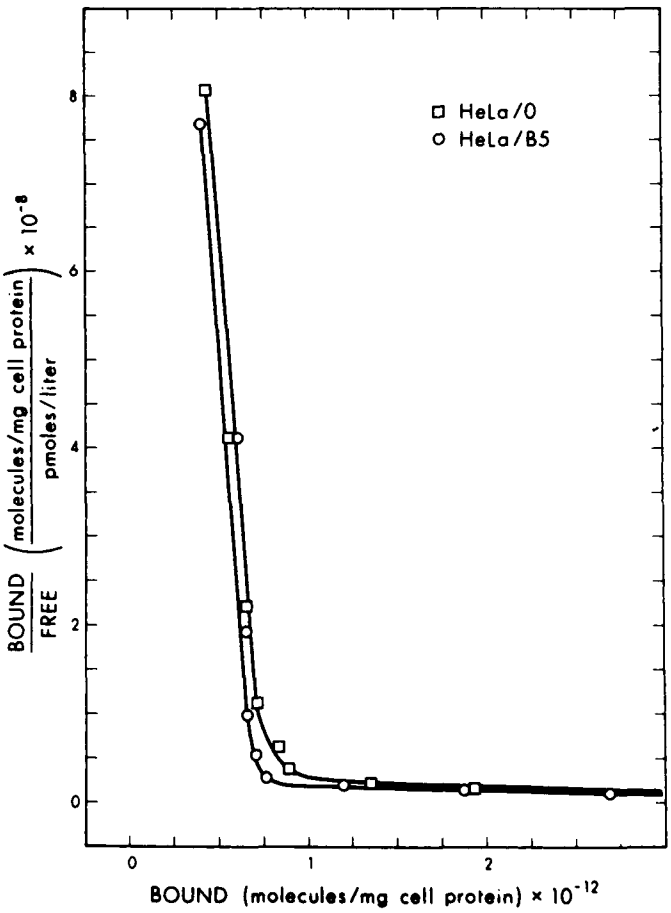

Fig. 5. Scatchard plots of NBMPR binding by HeLa/0 and HeLa/B5 cells. Replicate monolayer cultures were exposed at 20° for 5 min to 4 ml MEM-T medium containing various concentrations of [^{35}S]NBMPR; the [^{35}S] NBMPR-content of medium samples and of monolayers (after 1 wash with cold, buffered saline) was then determined.

protein*, respectively. Dissociation constants calculated from these data were 4.2×10^{-10} and 4.4×10^{-10}M for HeLa/0 and HeLa/B5 cells, respectively. These results indicate that the decreased responsiveness of HeLa/B5 cells to inhibition of thymidine uptake by NBMPR was not due to alterations in number of NBMPR binding sites, nor in the affinity of such sites for transport inhibitor.

DISCUSSION

Two types of BUdR resistance arising from decreased cellular uptake of BUdR have been found in animal cells: a) resistance attributable to a deficiency in thymidine kinase

*Recent studies in this laboratory (A.R.P. Paterson and C.E. Cass) have shown that the number of NBMR binding sites per HeLa cell is higher in S phase than in other cell cycle phases. Thus, values reported here for asynchronous cell populations represent the average of the NBMPR binding capacities of cells in the different cell cycle states.

activity has been the more frequently studied (14–17) and b) resistance due to impairment of the transport mechanism by which BUdR enters the cell has also been reported (18, 19). In the present study, the latter mechanism appears to account for BUdR/FUdR resistance in HeLa/B5 cells; we suggest that a reduced affinity of the thymidine transport mechanism for thymidine and thymidine analogs is responsible for low rates of uptake of the latter by HeLa/B5 cells, relative to those of HeLa/0 cells.

Two mechanisms for transport of nucleosides by animal cells have been described: a) the low affinity, low specificity systems found in erythrocytes (3–5) and in polymorphonuclear leucocytes (33) and b) the high affinity systems seen in various lines of cultured animal cells [for example, Novikoff hepatoma (1), HeLa (6–9), 3T3 (34)] which appeared to have distinct permeant specificities. Recent studies of thymidine uptake by ATP-depleted Novikoff hepatoma cells (10) have shown that these cells possess a low affinity transport mechanism with low specificity for nucleosides resembling the facilitated diffusion mechanism of human erythrocytes. In addition, studies from this laboratory of NBMPR inhibition of nucleoside uptake in HeLa cells have demonstrated the existence of NBMPR binding sites of a single class, occupancy of which results in inhibition of 4 functionally distinct nucleoside uptake mechanisms (6–9, 23).

BUdR and FUdR evidently enter HeLa cells by means of the thymidine transport mechanism and are phosphorylated by thymidine kinase. That the decreased uptake of these agents by HeLa/B5 cells was not a consequence of changes in thymidine kinase activity was indicated by the following: a) the cellular content of thymidine kinase activity was similar in HeLa/B5 cells and HeLa/0 cells, b) similar kinetic constants for thymidine kinase were obtained for both cell types, and c) substrate affinities for this enzyme were apparently comparable in both cell types because thymidine kinase elution profiles were similar when extracts of either cell type were chromatographed on thymidine kinase affinity gels.

Because NBMPR was known to interact in a highly specific manner with the nucleoside transport mechanism(s) of HeLa cells (6–9, 23), NBMPR was employed as a probe of the apparent alteration in thymidine transport in HeLa/B5 cells. Although thymidine uptake in both HeLa/0 and HeLa/B5 cells was less than 10% of control values in the presence of 10–12 μM NBMPR, at lower NBMPR concentrations thymidine uptake by HeLa/B5 cells was markedly less sensitive than that of HeLa/0 cells to inhibition by NBMPR. Sensitivity to NBMPR of adenosine, guanosine, and uridine uptake was comparable in both cell types. These results, together with those of the kinetic studies of thymidine uptake here reported, indicate that the activity of the thymidine transporter is altered in the variant cells. The relationship between the NBMPR binding site and the thymidine transporter is not understood, apart from the fact that NBMPR occupancy of the former prevents operation of the latter. The comparison of NBMPR binding by HeLa/0 and HeLa/B5 cells reported here indicates that the numbers of cellular binding sites and the dissociation constants for bound NBMPR were similar in both cell types. These findings suggest that HeLa/B5 cells are resistant to BUdR and FUdR because some aspect of the thymidine transporter function is defective.

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