PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 179, 463–471 (1985)

Genetic Analysis of the 6-Thiobenzylpurine Binding Site of the Nucleoside Transporter in Mouse Lymphoblasts (42124)

BRUCE ARONOW AND BUDDY ULLMAN

Department of Biochemistry, University of Kentucky Medical Center, Lexington, Kentucky 40536-0084

Abstract. From a mutagenized population of wild-type S49 T lymphoblasts, cells were selected for their ability to survive in semisolid medium containing 0.5 m*M* hypoxanthine, 0.4 μM methotrexate, 30 μM thymidine, 30 μM deoxycytidine, and 30 μM *p*-nitrobenzyl-6-thioinosine (NBMPR), a potent inhibitor of nucleoside transport. Unlike wild-type parental cells, two mutant clones, KAB1 and KAB5, were still sensitive to nucleoside-mediated cytotoxicity in the presence of NBMPR. Comparisons of the abilities of wild-type cells, KAB1, and KAB5 cells to incorporate exogenous nucleoside to the corresponding nucleoside triphosphate indicated that nucleoside incorporation was much less sensitive to inhibition by NBMPR in the mutant cells. Rapid transport studies indicated that the mutant cell lines, unlike the wild-type parent, had acquired an NBMPR-insensitive nucleoside transport component which was similar to the NBMPR-sensitive wild-type transporter with respect to affinities for nucleosides and sensitivities toward *N*-ethylmaleimide and dipyridamole. Binding studies with [^{3}H]NBMPR indicated that KAB5 cells were 70–75% deficient in the number of NBMPR binding sites, whereas KAB1 cells possessed a wild-type complement of NBMPR binding sites with wild-type binding characteristics. These data suggest that the NBMPR binding site in wild-type S49 cells is genetically distinguishable from the nucleoside carrier site and that the former may be a regulatory site.

Many nucleosides and synthetic nucleoside analogs are growth inhibitory and cytotoxic to mammalian cells and tissues. In order to exert many of their physiological and cytotoxic effects, nucleosides must permeate the plasma membrane of these cells. This nucleoside transport is carrier mediated and is independent from the subsequent intracellular metabolism of nucleosides. In the past there has existed conflicting evidence about the substrate specificity and multiplicity of these nucleoside carrier systems. The first evidence for a single nonspecific nucleoside carrier in animal cells was obtained from transport studies in human erythrocytes (1) and rabbit polymorphonuclear leukocytes (2) indicating the existence of a single saturable transport system for all purine and pyrimidine ribonucleosides and deoxyribonucleosides. Perhaps the most definitive evidence for a single nucleoside transport system came from the isolation and characterization of mutant lymphoma cells genetically deficient in nucleoside transport capability (3, 4). These cells are incapable of transporting virtually all exogenous nucleosides across the plasma membrane and their characterization provided conclusive evidence that the transport of all nucleosides requires a common component.

The study of nucleoside transport in mammalian cells has been greatly enhanced by the existence of specific high affinity inhibitors of the nucleoside transporter in mammalian cells. 4-Nitrobenzyl-6-thioinosine (NBMPR) is a competitive inhibitor of nucleoside transport which binds to mammalian cell plasma membranes with an apparent K_d around 0.1–1.0 n*M* (5–10). Not all cells, however, possess high affinity NBMPR binding sites or nucleoside transporters which are completely sensitive to NBMPR (8, 10–14). Certain mammalian cell lines possess NBMPR-insensitive components ranging from 2 to 100% of the total nucleoside transport (8, 10, 11, 13, 14). Moreover, nucleoside transport-deficient cells lack high affinity NBMPR binding sites (15). Whether the NBMPR-sensitive and NBMPR-insensitive nucleoside transporters are coded by a single gene or multiple genetic loci is unknown. To attempt to resolve whether mammalian cells possess one or two transporters with different NBMPR sensitivities, we have exploited the high affinity

0037-9727/85 $1.50

interaction of NBMPR with the nucleoside transport function in S49 murine lymphoma cells to isolate mutants resistant to the physiological effects of NBMPR. Growth rate determinations, incorporation studies, rapid transport measurements, and binding site quantitations indicated that mutant cells were abnormally responsive to NBMPR and had gained an NBMPR-insensitive nucleoside transporter function. The results of these studies may ultimately have important chemotherapeutic implications in the clinical utilization of transport inhibitors. Some of these uses include the potentiation of the effects of inhibitors of nucleotide metabolism in cancer cells (16, 17) or the modulation of nucleoside cytotoxicity in tumor cells (18–21) or pathogenic parasites (22, 23).

Materials and Methods. *Cell culture.* The S49 cell line is a mineral oil induced T-cell lymphocytic lymphoma originally derived from a Balb/c mouse (24). The growth characteristics and lymphocytic properties of wild-type S49 cells have been described previously in great detail (24, 25).

Selection of mutants. The rationale and methodologies for the selection of mutants resistant to the physiological effects of NBMPR have been described elsewhere (26). Two clones, KAB1 and KAB5, were selected from 10^6 wild-type cells in soft agarose containing 0.5 m*M* hypoxanthine, 0.4 μM methotrexate, 30 μM thymidine, 30 μM deoxycytidine, and 30 μM NBMPR.

Growth rate determinations. The growth sensitivities of wild-type and mutant cells to various nucleosides and nucleoside analogs were determined as described previously (3, 4, 27).

Incorporation of exogenous nucleoside into nucleotides. The abilities of wild-type and mutant cells to incorporate exogenous nucleoside from the culture medium in the presence or absence of inhibitors of nucleoside transport were determined as follows. Cells were preincubated at 37°C under normal growth conditions in the absence or presence of varying concentrations of the inhibitor of nucleoside transport. After a 20-min preincubation, cells were exposed for 4 hr to either 1 m*M* cytidine, 0.2 m*M* thymidine, 0.2 m*M* deoxyguanosine, or 0.1 m*M* deoxyadenosine in the presence of 10 μM erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) to prevent deamination of the deoxyadenosine by adenosine deaminase activity (28). Cells were harvested by centrifugation, and the culture medium carefully removed. The intracellular nucleotides were extracted by the method of Khym (29) as modified by Aronow *et al.* (30).

The nucleotides were separated and quantitated by high pressure liquid chromatography on a Whatman (Clifton, N.J.) Partisil strong anion exchange column using 0.4 *M* ammonium phosphate, pH 3.6, at a flow rate of 1.5 ml/min as the mobile phase (30).

Influx measurements over short time intervals. To assay nucleoside influx during short time intervals, cells were harvested by centrifugation and resuspended at a density of 2×10^7 cells/ml in fresh cell culture medium containing 20 m*M* Hepes, pH 7.4. These measurements were performed at 37°C and always within 30 min after the cells were transferred to the transport medium. Resuspended cells were pretreated for 20 min with inhibitors of nucleoside transport at various concentrations and nucleoside uptake measured as follows. One hundred microliters of medium containing 10 μCi/ml of the nucleoside permeant at various specific activities was layered over 100 μl of a silicone oil: paraffin oil/94:6 mixture (specific gravity of 1.03 g/ml) in a 1.5-ml polypropylene microfuge tube. Uptake measurements were initiated by the rapid addition of 100 μl of the cell suspension to the aqueous phase overlaying the organic layer. The assays were terminated by sedimenting the cells at 10,000*g* for 30 sec through the inert oil. After pelleting the cells, the radioactive upper layer was carefully removed from the microfuge tubes and the surface of the oil rinsed two times with 0.5 ml of PBS. After removal of the final PBS rinse and most of the silicone oil–paraffin oil mixture, the cell pellets were extracted with 0.1 ml of 2% Triton X-100. To the lysed and resuspended pellets 1.0 ml of scintillation fluid was added directly. The Eppendorf tubes were then placed in shell vials and the cell-associated radioactivity measured by liquid scintillation. In this fashion the translocation of nucleoside could be ascertained over 2-sec intervals. These short-term uptake measurements are subsequently

referred to in this manuscript as transport measurements. Transport measurements were performed in duplicate over a time course of 5–20 sec in the absence of NBMPR. In the presence of 10 μM NBMPR these measurements were performed for 1–3 min for wild-type cells to ensure sufficient entry of quantifiable radioactivity.

Measurements of 4-nitrobenzylthioinosine binding. The binding of [^{3}H]NBMPR to wild-type and mutant cells was measured by the glass-fiber filter disk procedure of Aronow *et al.* (26).

Results. *Selection and isolation of mutant clones.* Nucleoside transport in animal cells can be inhibited by a variety of compounds (31, 32). To dissect genetically the high affinity interactions of these compounds with the nucleoside transporter, we attempted to devise selective procedures to isolate variants resistant to the physiological effects of these inhibitors. Since the cytotoxic effects of 0.4 μM methotrexate in the presence of 0.5 mM hypoxanthine can be reversed by 16 μM thymidine, a pyrimidine nucleoside, the ability of various inhibitors of nucleoside transport to prevent thymidine rescue of methotrexate toxicity was determined. Maximum reversal of methotrexate-induced cytotoxicity in the absence of inhibitors of nucleoside transport occurred around 16 μM thymidine. Lower concentrations of exogenous thymidine could not provide sufficient thymidylate to achieve and maintain normal rates of DNA synthesis. Higher thymidine concentrations were cytotoxic. Wild-type cells in the presence of 10 μM NBMPR did not survive in hypoxanthine–methotrexate–thymidine (Fig. 1), whereas growth and survival in HAT medium was surprisingly unimpaired by either dipyridamole (33) or dilazep (34), two other transport inhibitors, at 10 μM concentrations. Thus the latter drugs permitted sufficient thymidine entry to allow for cell survival in the presence of amethopterin. A selective strategy was therefore devised by which NBMPR-insensitive clones could be isolated in medium containing 0.5 mM hypoxanthine, 0.4 μM methotrexate, 30 μM thymidine, 30 μM deoxycytidine, and 30 μM NBMPR.

Two clones, KAB1 and KAB5, were characterized further. Since KAB1 and KAB5 cells were isolated in semisolid selective medium, the abilities of these cells to survive in HAT medium containing 10 μM NBMPR in suspension culture were examined. As demonstrated in Fig. 1, wild-type parental cells could not survive in HAT medium in the presence of 10 μM NBMPR, while the KAB1 and KAB5 cells grew efficiently in HAT medium regardless of whether NBMPR was present. In similar experiments, it was demonstrated that thymidine salvage under HAT conditions in wild-type cells could be blocked at NBMPR concentrations as low as 0.3 μM. The possibility that the basis for this HAT–NBMPR resistance was due to more rapid degradation of the NBMPR by the KAB1 and KAB5 cells could be eliminated from the results of experiments in which culture medium containing 10 μM NBMPR incubated with mutant cells still killed wild-type cells in the presence of HAT (data not shown). The altered responsiveness of KAB1 and KAB5 cells to NBMPR was also reflected in their behavior toward two structural ana-

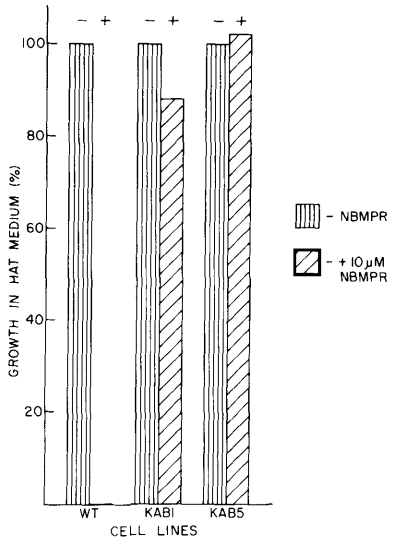

FIG. 1. Ability of wild-type and mutant cells to survive HAT medium in the absence or presence of NBMPR. The ability of wild-type, KAB1, and KAB5 cells to grow in culture medium containing 0.5 mM hypoxanthine, 0.4 μM methotrexate, and 16 μM thymidine were determined in the absence (▥) or presence (▨) of 10 μM NBMPR. The cells were counted after 72 hr and the results processed as described under Materials and Methods.

logs of NBMPR which are also strong inhibitors of the mammalian nucleoside transport system (35), 4-nitrobenzylthioguanosine (NBTGR) and 2-hydroxy-4-nitrobenzylthioinosine (HNBMPR). Both NBTGR and HNBMPR prevented the growth of wild-type cells but had virtually no effect on the abilities of KAB1 and KAB5 cells to grow in HAT medium (data not shown).

To determine whether the altered apparent sensitivities of KAB1 and KAB5 cells to NBMPR were specific for thymidine or general for a spectrum of nucleosides, the ability of various NBMPR concentrations to protect wild-type and mutant cells from the cytotoxic effects of 6-thioguanosine (Fig. 2A), deoxyadenosine in the presence of 10 μM EHNA (Fig. 2B), deoxyguanosine (data not shown), 5-fluorouridine (data not shown), and adenosine in the presence of 10 μM EHNA (data not shown) were examined. Wild-type and mutant cells were approximately equally sensitive to any of the five nucleosides in the absence of NBMPR. For all five nucleosides, NBMPR protected wild-type cells from the nucleoside-mediated cytotoxicity, and therefore the effective concentration of nucleoside which inhibited growth by 50% (EC_{50} value) was increased by NBMPR. After plotting the EC_{50} value for a specific drug as a function of NBMPR concentration, it is apparent that the KAB1 and KAB5 cell lines were altered in their ability to be protected from nucleoside toxicity by NBMPR, Fig. 2. Conversely, 10 μM (Fig. 2) and lower (data not shown) concentrations of dipyridamole in the absence of NBMPR protected all three cell lines equally from the nucleoside-mediated growth inhibition. Thus, the altered responsiveness of KAB1 and KAB5 cells to NBMPR appeared to be a general phenomenon for all nucleosides but limited to NBMPR and not to other inhibitors of nucleoside transport.

Incorporation of nucleosides. The altered responses of KAB1 and KAB5 cells to NBMPR protection of nucleoside cytotoxicity suggested that NBMPR failed to prevent nucleoside entry in these mutants. Thus, the abilities of wild-type and mutant cells to incorporate exogenous nucleoside into nu-

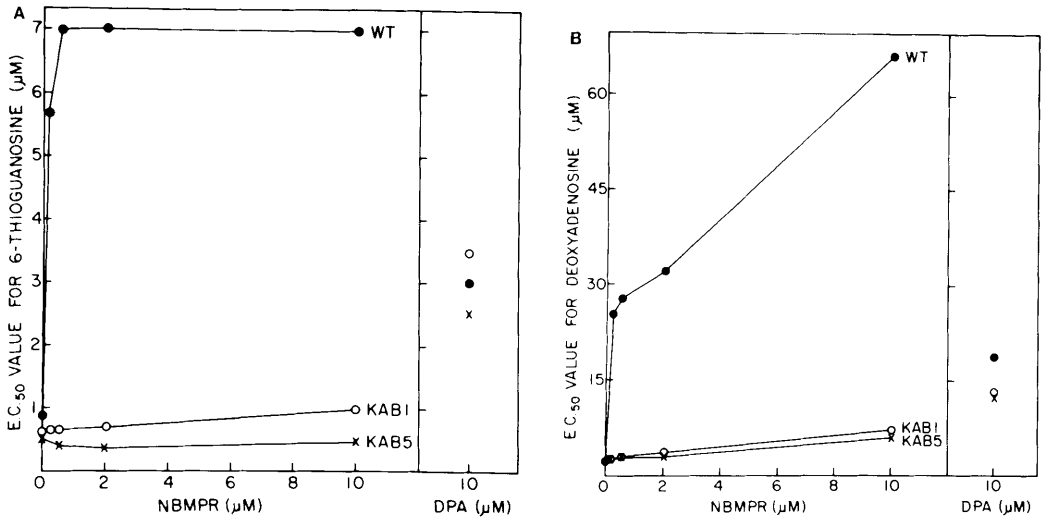

FIG. 2. Effect of NBMPR on nucleoside cytotoxicity in wild-type and mutant cells. The cytotoxicities of 6-thioguanosine (A), and deoxyadenosine (B) were examined as a function of NBMPR concentration for wild-type (●), KAB1 (○), and KAB5 (×) cells. Erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA; 10 μM) was added to the growth experiments containing deoxyadenosine to prevent deamination. The data are plotted to indicate the effective concentration of nucleoside which inhibited growth by 50% (EC_{50} value) as a function of NBMPR concentration. These experiments were repeated several times and the results of a typical experiment are depicted. The effects of 10 μM dipyridamole on nucleoside toxicity in the absence of NBMPR are also shown.

cleoside triphosphate pools in the absence and presence of NBMPR were compared. As demonstrated in Fig. 3, both KAB1 and KAB5 cells in the presence of NBMPR accumulated 1 m*M* cytidine into CTP and 0.1 m*M* deoxyadenosine into dATP much more efficiently than wild-type cells. Similar results were obtained with deoxyguanosine and thymidine (data not shown). AE_1 cells which are genetically deficient in nucleoside transport capability (3) failed to accumulate either nucleoside into the corresponding nucleoside triphosphate (Fig. 3). The effects of 10 μM dipyridamole on nucleoside incorporation were comparable for all three cell lines for all nucleosides (data not shown).

Influx of nucleosides over short time intervals. The measurements of the effects of NBMPR on nucleoside incorporation into cellular nucleotide pools reflected both transport and metabolic components. Thus, the rates of influx of [^{3}H]cytidine (Fig. 4) and [^{3}H]adenosine (data not shown) were measured in wild-type and mutant cells in the absence (Fig. 4A) and presence (Fig. 4B) of 10 μM NBMPR over short time intervals. Transport of both nucleosides into wild-type cells could be virtually completely (>99.5%) blocked by 10 μM NBMPR. In KAB1 and KAB5 cells, however, transport was refractory to complete inhibition by NBMPR. Approximately 5–20% of the total nucleoside transport capacity of KAB1 and KAB5 cells was insensitive to inhibition by 10 μM NBMPR, Fig. 4. The responses of the mutant cells to inhibition of nucleoside transport by NBMPR suggested that the nucleoside transporter in mutant cells had gained an NBMPR-insensitive component not observed in wild-type parental cells. This NBMPR-insensitive nucleoside transport component of KAB1 and KAB5 cells had approximately the same apparent K_m values for adenosine and cytidine as the NBMPR-sensitive component, Table I.

Nitrobenzylthioinosine binding sites. To elucidate the nature of the mutations in KAB1 and KAB5 cells at the molecular level,

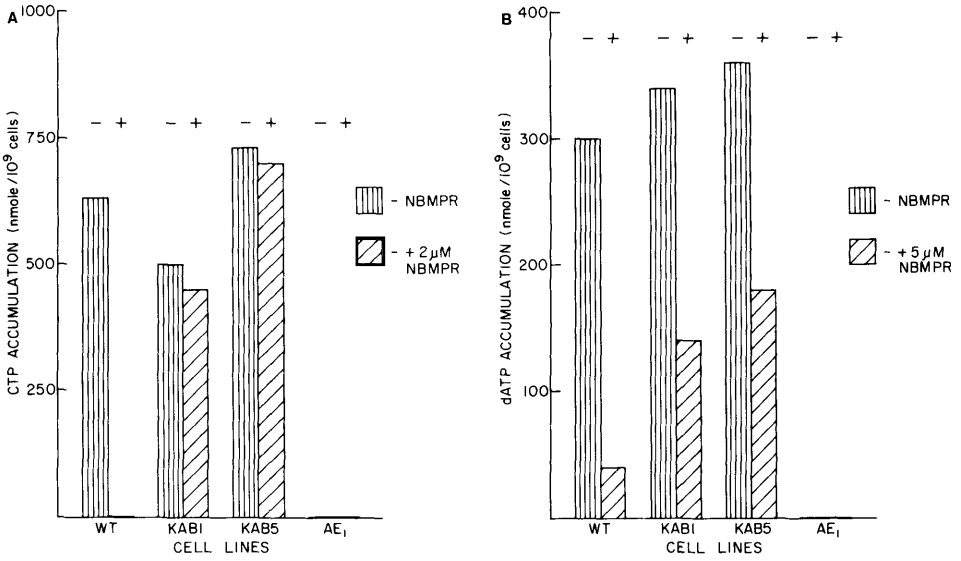

FIG. 3. Effects of NBMPR on incorporation of exogenous nucleosides into intracellular nucleoside triphosphates. The abilities of wild-type, KAB1, and KAB5 cells to incorporate either 1 m*M* cytidine into CTP (A) or 0.1 m*M* deoxyadenosine into dATP (B) in the absence (▥) or presence (▨) of NBMPR were determined after 4-hr incubations under normal growth conditions. 10 μM EHNA was added to all S49 cells prior to the addition of deoxyadenosine to inhibit adenosine deaminease activity. Nucleoside triphosphate concentrations were determined by high performance liquid chromatography as described by Aronow *et al.* (30). The results are those of a single typical experiment which has been repeated one other time with similar results.

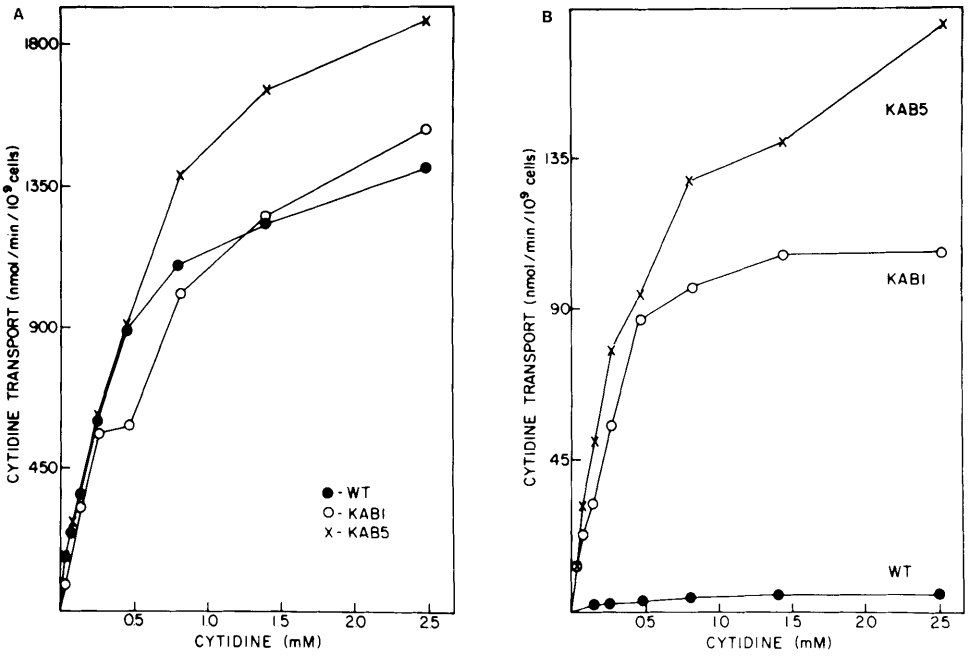

FIG. 4. Cytidine transport in wild-type and mutant cells. The abilities of wild-type (●), KAB1 (O), and KAB5 (×) cells to transport cytidine were determined as a function of concentration in the absence (A) and presence (B) of 10 μM NBMPR. Initial transport rates were determined after 5 and 10-sec intervals without NBMPR and between 180 and 300 sec for NBMPR-treated cells. The exact intervals depended on the rate so as to be linear with time. Experiments were performed as described under Materials and Methods at 37°C, and the results are those of a single typical experiment which has been repeated three times with very similar results.

the NBMPR binding sites were evaluated on wild-type and mutant cells (Fig. 5). The results demonstrated clear and consistent differences between the KAB1 and KAB5 cell lines. Figure 5 indicates that wild-type and KAB1 cells had equivalent numbers of NBMPR binding sites, while KAB5 cells possessed about one-third of the wild-type complement of NBMPR binding sites. A fourth cell line, AE_1 (3), which is genetically deficient in nucleoside transport, did not contain any measurable high affinity [^{3}H]-NBMPR binding sites (15). In the experiment depicted in Fig. 5, the dissociation constants for NBMPR with the receptors on the surface of wild-type, KAB1, and KAB5 cells were determined to be about 0.5, 0.5, and 0.3 nM, respectively. Nonradiolabeled nucleosides and dipyridamole displaced [^{3}H]NBMPR from the NBMPR receptor with equal efficacy in all three cell lines (data not shown).

TABLE I. APPARENT K_m VALUES OF THE NUCLEOSIDE TRANSPORTER FOR NUCLEOSIDES[a]

	K_m values (μM)			
	Adenosine		Cytidine	
	NBMPR		NBMPR	
Cell line	−	+	−	+
Wild type	25	nd	400	nd
KAB1	22	30	650	280
KAB5	22	58	550	450

[a] The apparent K_m values for the transport of [^{3}H]adenosine and [^{3}H]cytidine were determined from the X axis intercepts of double reciprocal plots. The NBMPR concentration was 10 μM. Not determinable, nd.

Discussion. Based on a variety of experimental observations, Jarvis and Young (36) and Jarvis *et al.* (37) have proposed that NBMPR and nucleosides bind to a common

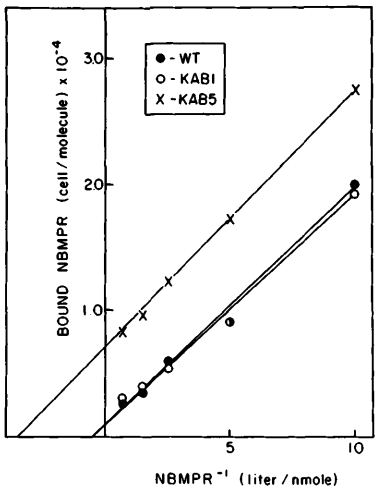

FIG. 5. NBMPR binding to wildtype and mutant S49 cells. The interactions of [^{3}H]NBMPR with 7×10^6 wild-type (●), KAB1 (○), and KAB5 (×) cells were measured in 1.0 ml vol with increasing concentrations of [^{3}H]NBMPR (16 Ci/mmole). This experiment has been repeated 5–10 other times with each cell line, and the results reported are those of a single typical experiment.

permeation site. First, NBMPR and naturally occurring nucleosides are similar structurally. Second, NBMPR inhibits nucleoside transport in a competitive fashion (15, 36, 37). Third, nucleosides displace [^{3}H]NBMPR from the surface of animal cells in a competitive fashion (15, 38). Fourth, the number of NBMPR binding sites in red blood cells among mammalian species is linearly proportional to the total nucleoside transport capacity (36, 37). Finally, Cass *et al.* (15) have demonstrated that S49 cells genetically deficient in nucleoside transport have lost their ability to bind radiolabeled NBMPR, and Jarvis and Young (39, 40) have shown that nucleoside impermeable sheep erythrocytes possess no NBMPR binding sites.

Nevertheless, whether NBMPR binds to the nucleoside substrate site, albeit with much higher affinity, or to a second site, has not been definitively elucidated. In these S49 cells (15) and in human erythrocytes (5, 12, 35, 38, 41), NBMPR binding abolishes nucleoside transport. In other cell lines, however, the extent to which NBMPR inhibits the transport of nucleosides varies considerably. For instance, uridine transport into Walker 256 rat carcinoma cells (13) and Novikoff hepatoma cells (10), as well as thymidine transport into the latter cells (12), are refractory to inhibition by NBMPR. Both Walker carcinoma cells (42) and Novikoff N1S1-67 hepatoma cells (10) have been reported to lack NBMPR binding sites.

Moreover, some cells possess both NBMPR-sensitive and NBMPR-insensitive nucleoside transport components. Belt indicated that uridine permeation is inhibited maximally by NBMPR by 98, 90, and 80% in RPMI1640, P388, and L1210 cells, respectively (13, 14). Plagemann and Wohlheuter have demonstrated that uridine influx in CHO, P388, L1210, and L929 cells behaves in a biphasic manner with respect to NBMPR inhibition, each cell line containing an NBMPR-sensitive and an NBMPR-insensitive component (10). Thymidine transport, as well, is not completely inhibited by NBMPR in CHO, L, P388, and HeLa cells (12, 43). Important questions therefore need to be resolved concerning whether NBMPR-sensitive and NBMPR-insensitive transporters are genetically identical except for their ability to bind and interact with NBMPR and related compounds and whether the nucleoside binding site and the NBMPR binding site are the same.

To address these questions, the nucleoside transporter function in S49 cells has been genetically manipulated to isolate not so rare variants which had gained an NBMPR-insensitive nucleoside transporter function. It appears that two types of mutants possessing an NBMPR-insensitive transporter can be generated. KAB5 cells lost over half of their NBMPR binding sites and could still transport nucleosides as efficiently as wild-type cells. This then was a genetic deficiency in NBMPR binding that did not result in decreased nucleoside transport. KAB1 cells on the other hand possessed an NBMPR binding site which did not differ from the NBMPR binding site of wild-type cells with respect to multiple kinetic parameters. Yet KAB1 cells possessed an NBMPR-resistant nucleoside transport function. Thus, a proportion of the NBMPR binding sites in the KAB1 cell line were uncoupled by mutation from the nucleoside permeation site. This mutation presumably would be in some genetically deter-

mined transducing function required for linking NBMPR binding to its inhibitory effects on nucleoside transport. These data and those described by Aronow *et al.* (26) support the existence of a single nucleoside transporter which can probably exist in two conformations, either coupled or uncoupled to the NBMPR binding site in a genetically alterable fashion. Moreover, the accumulated data strongly suggest that NBMPR binds to the nucleoside transporter at a site distinct from the nucleoside binding site.

The NBMPR-insensitive transporter is still sensitive to dipyridamole, however. The effects of dipyridamole on nucleoside transport in S49 cells are not as complete as those of NBMPR. For instance, dipyridamole does not protect wild-type S49 cells from nucleoside toxicity (Fig. 2) as much as NBMPR and still allows sufficient thymidine incorporation into TTP (personal observations) to allow cells to survive HAT–dipyridamole medium. These data are contrasted by those of Van Mouwerik *et al.* (44) in which the toxicity of amethopterin toward cultured human colon cells is enhanced by dipyridamole-mediated inhibition of thymidine salvage. The differential findings between S49 and HCT 116 (44) cells probably reflects the extent to which dipyridamole inhibits thymidine transport. In nucleoside transport-deficient S49 cells (3), a 95% decrease in thymidine transport does not decrease thymidine incorporation into TTP or thymidine cytotoxicity, since the former assays are performed over 2-sec intervals, while the latter are performed over hours or days. Nucleoside incorporation and toxicity can only be inhibited under conditions in which transport rather than metabolism becomes rate limiting. With respect to thymidine transport in S49 cells, a >95% deficiency in thymidine transport is required, which can be pharmacologically induced by NBMPR but not by dipyridamole or a genetic deficiency in the nucleoside transport function.

The authors thank Ms. Lisa Miller, Ms. Donna Wilkie, and Ms. Ginger Hodges for their help in the preparation of this manuscript. This work was supported by Grant R01 CA32580 from the National Institutes of Health. B.U. is a recipient of a Research Career Development Award from the National Institutes of Health.

1. Oliver JM, Paterson ARP. Nucleoside transport. I. A mediated process in human erythrocytes. J Biol Chem **49**:262–270, 1971.
2. Taube RA, Berlin RD. Membrane transport of nucleosides in rabbit polymorphonuclear leukocytes. Biochim Biophys Acta **255**:6–18, 1972.
3. Cohen A, Ullman B, Martin DW Jr. Characterization of a mutant mouse lymphoma cell with deficient transport of purine and pyrimidine nucleosides. J Biol Chem **254**:112–116, 1979.
4. Ullman B, Kaur K, Watts T. Genetic studies on the role of the nucleoside transport function in nucleoside efflux, the inosine cycle, and purine biosynthesis. Mol Cell Biol **3**:1187–1196, 1983.
5. Pickard MA, Brown RR, Paul B, Paterson ARP. Binding of the nucleoside transport inhibitor 4-nitrobenzylthioinosine to erythrocyte membranes. Canad J Biochem **51**:666–672, 1973.
6. Cass CE, Gaudette LA, Paterson ARP. Mediated transport of nucleosides in human erythrocytes. Specific binding of the inhibitor nitrobenzylthioinosine to nucleoside transport sites in the erythrocyte membrane. Biochim Biophys Acta **345**:1–10, 1971.
7. Lauzon GJ, Paterson ARP. Binding of the nucleoside transport inhibitor nitrobenzylthioinosine to HeLa cells. Mol Pharmacol **13**:883–891, 1977.
8. Dahlig-Harley E, Eilam Y, Paterson ARP, Cass CE. Binding of nitrobenzylthioinosine to high-affinity sites on the nucleoside transport mechanism of HeLa cells. Biochem J **200**:295–305, 1981.
9. Wohlheuter RM, Brown WE, Plagemann PGW. Kinetic and thermodynamic studies on nitrobenzylthioinosine binding to the nucleoside transporter of Chinese hamster ovary cells. Biochim Biophys Acta **731**:168–176, 1983.
10. Plagemann PGW, Wohlheuter RM. Nucleoside transport in cultured mammalian cells. Multiple forms with different sensitivity to inhibition by nitrobenzylthioinosine or hypoxanthine. Biochim Biophys Acta **773**:39–52, 1984.
11. Eilam Y, Cabantchik ZI. The mechanism of interaction between high-affinity probes and the uridine transport system of mammalian cells. J Cell Physiol **89**:831–838, 1977.
12. Wohlheuter RM, Marz R, Plagemann PGW. Properties of the thymidine transport system of Chinese hamster ovary cells as probed by nitrobenzylthioinosine. J Membr Biol **42**:247–264, 1978.
13. Belt JA. Nitrobenzylthioinosine-insensitive uridine transport in human lymphoblasts and murine leukemia cells. Biochem Biophys Res Commun **110**: 417–412, 1983.
14. Belt JA. Heterogeneity of nucleoside transport in mammalian cells. Two types of transport activity in L1210 and other cultured neoplastic cells. Mol Pharmacol **24**:479–484, 1983.
15. Cass CE, Kolassa N, Uehara Y, Dahlig-Harley E, Harley ER, Paterson ARP. Absence of binding sites

for the transport inhibitor nitrobenzylthioinosine on nucleoside transport-deficient mouse lymphoma cells. Biochim Biophys Acta **649**:769–777, 1981.
16. King ME, Howell SB. Inhibition of uridine uptake and potentiation of PALA cytoxicity by dipyridamole *in vitro.* Proc Amer Assoc Cancer Res **23**:207, 1982.
17. Zehn Y, Lui MS, Weber G. Effects of acivicin and dipyridamole on hepatoma 3924A cells. Cancer Res **43**:1616–1619, 1983.
18. Paterson ARP, Paran JH, Yang S, Lynch TP. Protection of mice against lethal dosages of nebularine by nitrobenzylthioinosine, an inhibitor of nucleoside transport. Cancer Res **39**:3607–3611, 1979.
19. Lynch TP, Paran JH, Paterson ARP. Therapy of mouse leukemia L1210 with combinations of nebularine and nitrobenzylthioinosine-5′-phosphate. Cancer Res **41**:560–565, 1981.
20. Lynch TP, Jakobs ES, Paran, JH, Paterson ARP. Treatment of mouse neoplasms with high dose tubercidin. Cancer Res **41**:3200–3204, 1981.
21. Paterson ARP, Kolassa N, Cass CE. Transport of nucleoside drugs in animal cells. Pharmacol Ther **12**:515–536, 1981.
22. Ogbunude POJ, Ikediobi CO. Effect of nitrobenzylthioinosinate on the toxicity of tubercidin and ethidium against *Trypanosoma gambiense.* Acta Trop **39**:219–224, 1982.
23. El Kouni MH, Diop D, Cha S. Combination therapy of schistosomiasis by tubercidin and nitrobenzylthioinosine 5′-phosphate. Proc Natl Acad Sci USA **80**:6667–6670, 1983.
24. Horibata K, Harris AW. Mouse myelomas and lymphomas in cell culture. Exp Cell Res **60**:61–77, 1970.
25. Ullman B, Cohen A, Martin DW Jr. Characterization of a cell culture model for the study of adenosine deaminase- and purine nucleoside phosphorylase-deficient immunologic disease. Cell **9**:205–211, 1976.
26. Aronow B, Allen K, Patrick J, Ullman B. Altered nucleoside transporters in mammalian cells selected for resistance to the physiological effects of inhibitors of nucleoside transport. J Biol Chem, 1985, in press.
27. Ullman B, Gudas LJ, Cohen A, Martin DW Jr. Deoxyadenosine metabolism and cytotoxicity in cultured mouse T-lymphoma cells: A model for immunodeficiency disease. Cell **14**:365–375, 1978.
28. Schaeffer JJ, Schwender CF. Enzyme inhibitor 26 bridging hydrophobic and hydrophilic regions on adenosine deaminase with some 9-(2-hydroxy-3-alkyl) adenines. J Med Chem **17**:6–8, 1974.
29. Khym JX. An analytical system for rapid separation of tissue nucleotides at low pressures on conventional anion exchangers. Clin Chem **21**:1245–1252, 1975.
30. Aronow B, Watts T, Lassetter J, Washtien W, Ullman B. Altered biochemical phenotype of a murine lymphoma cell with elevated CTP levels and altered CTP synthetase activity. J Biol Chem **259**:9035–9043, 1984.
31. Berlin RD, Oliver JM. Membrane transport of purine and pyrimidine bases and nucleosides in animal cells. Int Rev Cytol **42**:287–336, 1975.
32. Plagemann PGW, Wohlheuter RM. Permeation of nucleosides, nucleic acid bases, and nucleotides in animal cells. Curr Top Membr Transp **14**:225–330, 1980.
33. Scholtissek C. Studies on the uptake of nucleic acid precursors into cells in tissue culture. Biochim Biophys Acta **158**:435–447, 1968.
34. Fuijita S, Ishida Y, Izumi K, Moritoki H, Ohara M, Takei T. Potentiation by dilazep of the negative inotropic effect of adenosine on guinea-pig atria. Brit J Pharmacol **68**:343–349, 1980.
35. Paterson ARP, Oliver JM. Nucleoside transport. II. Inhibition by p-nitrobenzylthioguanosine and related compounds. Canad J Biochem **49**:271–274, 1971.
36. Jarvis SM, McBride D, Young JD. Erythrocyte nucleoside transport: Asymmetrical binding of nitrobenzylthioinosine to nucleoside permeation sites. J Physiol **324**:31–46, 1982.
37. Jarvis SM, Young JD. Nucleoside translocation in sheep reticulocytes and fetal erythrocytes: A proposed model for the nucleoside transporter. J Physiol **324**: 47–66, 1982.
38. Cass CE, Paterson ARP. Nitrobenzylthioinosine binding sites in the erythrocyte membrane. Biochim Biophys Acta **419**:285–294, 1976.
39. Jarvis SM, Young JD. Direct evidence for the specific interaction of nitrobenzylthioinosine with functional nucleoside transport sites in sheep erythrocyte membranes. J Physiol **284**:96P–97P, 1978.
40. Jarvis SM, Young JD. Nucleoside transport in human and sheep erythrocytes. Evidence that nitrobenzylthioinosine binds specifically to functional nucleoside-transport sites. Biochem J **190**:377–383, 1980.
41. Cass CE, Paterson ARP. Mediated transport of nucleosides in human erythrocytes. Accelerated exchange diffusion of uridine and thymidine and specificity toward pyrimidine nucleosides as permeants. J Biol Chem **247**:3314–3320, 1972.
42. Paterson ARP, Jakobs ES, Harley ER, Cass CE, Robins MJ. In: Chang YC, Goz B, eds. Development of Target Oriented Anticancer Drugs. New York, Raven Press, pp41–56, 1983.
43. Cass CE, Paterson ARP. Inhibition of thymidine uptake in asynchronous HeLa cells by nitrobenzylthioinosine. Exp Cell Res **105**:427–435, 1977.
44. Van Mouwerik TJ, Pangallo C, Willson JKV, and Fischer PH. Dipyridamole (dp) augments the cytotoxicity of methotrexate (mtx) against human colon cancer cells. Proc Amer Assoc Cancer Res **25**:335, 1984.

Received January 28, 1985. P.S.E.B.M. 1985, Vol. 179.
Accepted March 6, 1985.