

# Nucleoside transporter proteins

## From Membrane Biology to Therapeutic Applications

### The 2001 Jeanne Manery Fisher Memorial Lecture

June 2, 2001,  
Alliston, ON

Carol E. Cass,  
Departments of  
Oncology &  
Biochemistry  
Canada Research Chair  
in Oncology

CIHR Membrane  
Protein Research Group  
University of Alberta  
Department of  
Experimental Oncology  
Cross Cancer Institute  
11560 University Ave.  
Edmonton, Alberta  
T6G 1Z2  
Tel: 780-432-8320  
carol.cass@cancerboard.  
ab.ca

### Introduction

Because this award lecture, given in memory of Jeanne Manery Fisher, celebrates my lifetime research achievements, I will give a historical overview of the field of nucleoside transport, which coincides almost exactly with my academic career at the University of Alberta. I will trace the history of the nucleoside transport field, from our early functional studies in human erythrocytes to our current studies of nucleoside transporter proteins and their importance in nucleoside biology and therapeutics.

Nucleosides are central metabolites in all life forms and, as precursors of nucleotides, play an essential role in intermediary metabolism, biosynthesis of macromolecules and cell signaling through interaction with purinergic receptors. Nucleoside drugs are used to treat hematologic malignancies, certain solid tumors and many viral diseases. A natural nucleoside, 2'-deoxycytidine, and three of its analogs that have important applications in either antiviral therapy (lamivudine) or anticancer therapies (gemcitabine, cytarabine) are shown in Fig. 1.

Since most nucleosides are hydrophilic molecules and don't cross cell membranes readily by diffusion, cellular utilization of extracellular nucleosides is dependent on the activity of specialized membrane proteins that translocate organic solutes across lipid bilayers.

In humans, the physiologic nucleosides and most nucleoside drugs enter cells via one or more of the five known nucleoside transporter proteins (NT) that have been identified during the past decade by molecular cloning and functional expression of cDNAs encoding the NT proteins. Their characteristics will be described in detail later. All of the known nucleoside transporters play an important role in nucleotide metabolism by their catalysis of the first step in nucleoside "salvage" pathways and the bidirectional transporters also probably play an

important role in cellular release of nucleosides (Fig. 2).

### The beginning: studies of nucleoside transport in human erythrocytes

The story begins in the late 1960s, when Dr. A.R.P. Paterson (McEachern Cancer Research Laboratory, University of Alberta) discovered that uptake of uridine and thymidine by human erythrocytes was mediated by a process that exhibited the hallmark characteristics of facilitated diffusion (1, 2). I joined the Paterson research group as a postdoctoral fellow in 1970, just as the nucleoside transport project was gathering momentum. At the time, nucleoside analogs were being aggressively examined as potential anticancer drugs in the U.S. National Cancer Institute's drug discovery program and there was considerable interest in the possibil-

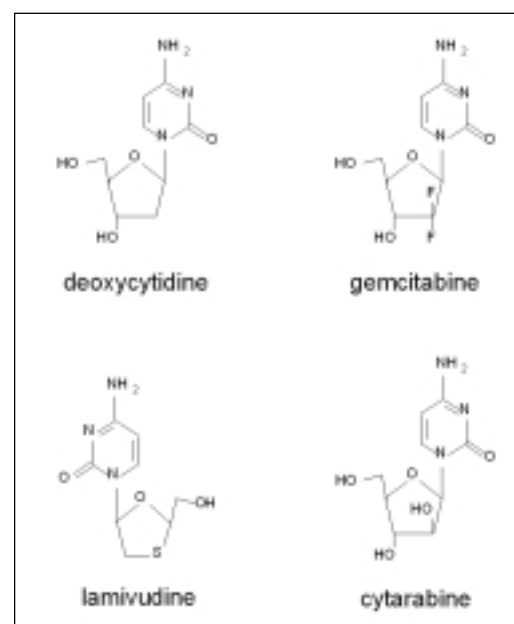


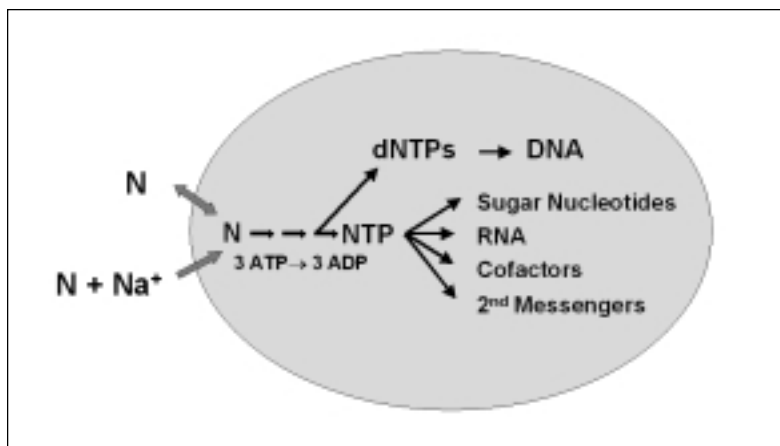
Figure 1. Deoxycytidine and analog drugs

ity that cellular uptake might be an important determinant of their pharmacologic activity.

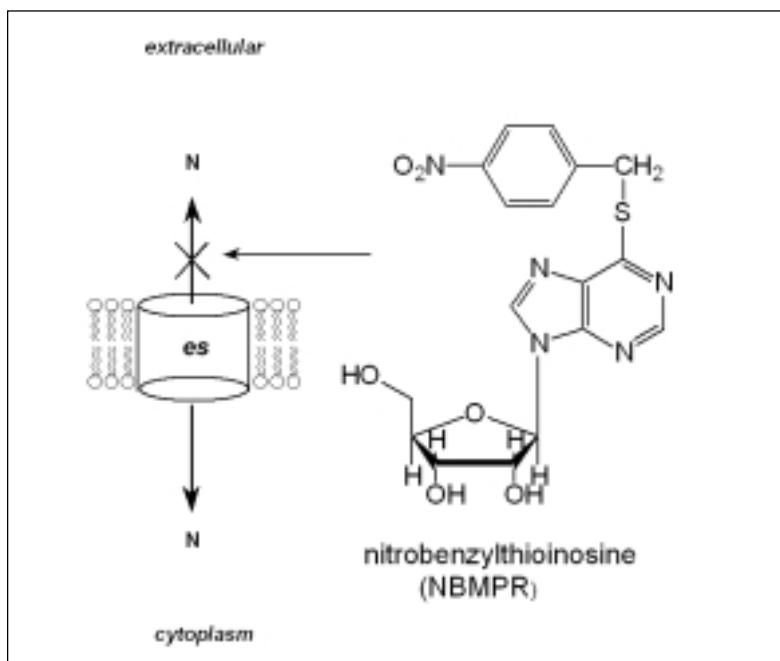
During the 1970s, the major focus of research was on the functional characterization of nucleoside transport processes. Human erythrocytes, which lack intracellular organelles, have only a single membrane type, thereby enabling analysis of processes of the plasma membrane without the necessity of membrane fractionation. Human erythrocytes also lack many enzymes of nucleotide metabolism, including the kinases required for salvage of uridine and thymidine, thereby enabling kinetic studies of nucleoside transport in the absence of metabolism. Because of these characteristics, erythrocytes of humans, and other species, were extensively used for functional characterization of nucleoside transport processes. Although we didn't know it at the time, human erythrocytes were also ideal for functional studies of nucleoside transport because they are one of the few human cell types that possess a single, rather than multiple, nucleoside transport processes. During the 1970s, the nucleoside transport process of human erythrocytes was shown to be equilibrative in nature, was characterized kinetically and was shown to exhibit broad permeant selectivity.

A key discovery, which subsequently led to the identification of equilibrative nucleoside transporter proteins, was the demonstration that a group of S-substituted thiopurine ribonucleosides, which had been synthesized as potential anticancer drugs, exhibit potent nucleoside-transport inhibitory activity in erythrocytes (2). We established that nitrobenzylthioinosine (NBMPR) binds to a single set of high-affinity sites ( $K_d$ , 1 nM;  $10^4$  sites/cell) on erythrocyte plasma membranes, the occupancy of which is directly correlated to inhibition of uridine transport (3-5). The erythrocytic nucleoside transporter has subsequently been extensively studied and is the prototypic equilibrative NBMPR-sensitive (*es*) transporter, now known through molecular cloning to be the hENT1 protein (Fig. 3).

We defined the broad substrate selectivity of the *es* transporter in kinetic studies in erythrocytes (6, 7) and subsequent work by ourselves and others extended these observations to erythrocytes from other species (4, 8), and as rapid-assay technologies were developed (9, 10), to more complex cell types, including cultured cancer cell lines (11-15). Our studies of the relative abilities of structural analogs of NBMPR to inhibit nucleoside transport in cells (14-19) and high-affinity binding of NBMPR in mem-



**Figure 2.** Role of plasma membrane transporters in nucleoside (N) metabolism in mammalian cells



**Figure 3.** NBMPR, a tight-binding inhibitor of the equilibrative sensitive (*es*) transporter

brane preparations (5, 18, 20-23) provided the basis for the wide-spread use of NBMPR as a molecular probe for quantification and identification of the *es* transporter protein in cell membranes. We also demonstrated that two potent, structurally unrelated inhibitors of nucleoside transport, dipyridamole and dilazep, inhibited binding of NBMPR to the *es* transporter (22, 24), thereby stimulating interest in the nucleoside-transport inhibitory capabilities of these, and related, compounds.

## Multiple nucleoside transport processes

During the 1980s, it became increasingly evident that mammalian cells possess multiple nucleoside transporter types, based on permeant selectivities, inhibition by diagnostic agents, and mechanisms of transport (for reviews, see 25, 26). We now know that these processes comprise two functionally distinct groups that differ in their fundamental mechanisms of transport. The equilibrative processes, which exhibit the classic features of a bidirectional, non-concentrative process, have been subdivided on the basis of their sensitivities to nanomolar concentrations of NBMPR into the *es* (equilibrative-sensitive) and *ei* (equilibrative-insensitive) processes. The *es* and *ei* processes transport a structurally diverse group of nucleosides and the *ei* process also transports nucleobases.

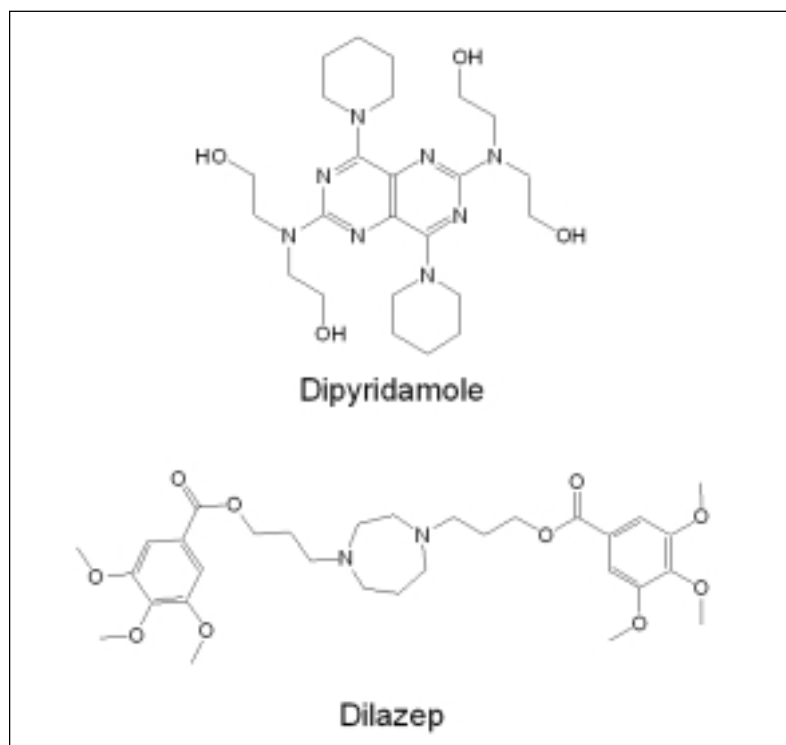
The concentrative processes are Na<sup>+</sup>-dependent, moving nucleosides into cells against their concentration gradients and have been subdivided into several functional groups. The *cit*, *cif* and *cib* processes are insensitive to NBMPR and accept, respectively, pyrimidine nucleosides, uridine plus purine nucleosides or pyrimidine and purine

nucleosides as permeants. Two minor processes (*csg*, *cs*), which are inhibited by NBMPR, have also been described.

## Transporter discovery by molecular cloning: two new membrane protein families

The nucleoside transporters of mammalian cells are hydrophobic membrane proteins of low-abundance that have been notoriously difficult to study biochemically. With the exception of the transporter of human erythrocytes, which was purified in very small quantities in the late 1980s (27), efforts to isolate the proteins responsible for various nucleoside-transport activities failed. Molecular cloning strategies became increasingly attractive as an approach to identify the elusive nucleoside transporter proteins. In 1990, we established a long-term collaboration with Dr. J.D. (Jim) Young (Physiology, University of Alberta) and Dr. S.A. (Steve) Baldwin (Biochemistry & Molecular Biology, University of Leeds) to identify nucleoside transporter proteins by molecular cloning. Because we had physical information about only one of an uncertain number of different nucleoside transporter subtypes, we invested heavily in strategies for functional expression selection of candidate cDNAs, recognizing that expression methods would be essential for the subsequent characterization of newly discovered nucleoside transporter proteins. The Young group focused on functional expression of nucleoside transporters in oocytes of *Xenopus laevis*, and their system has become the 'gold standard' for nucleoside transporter identification. We focused on the use of the yeast *Saccharomyces cerevisiae* and cultured mammalian cells for functional expression of nucleoside transporter proteins.

Identification of the first nucleoside transporter protein occurred in 1994 by functional-expression cloning in Dr. Young's laboratory, and its sequence revealed a new membrane protein family with representatives among bacteria (28). The next major breakthrough came in 1997 when our three-laboratory consortium successfully cloned a cDNA encoding the first recognized member of a second family of membrane proteins (29). Since then, our collaboration has been extraordinarily successful with the discovery of five nucleoside transporters from human cells, an equal number from rodents and several from lower organisms, including yeast, protozoan parasites, nematode worms and bacteria.

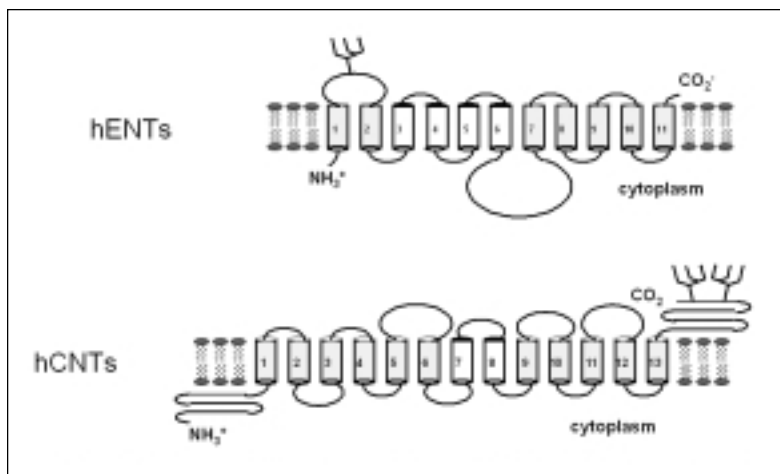


**Figure 4** Inhibitors of equilibrative nucleoside transport and NBMPR binding

The nucleoside transporter proteins are members of two evolutionarily old protein families: the Equilibrative Nucleoside Transporters (ENTs) and Concentrative Nucleoside Transporters (CNTs). cDNAs encoding representatives of the two ENT subfamilies (ENT1 and ENT2: exhibit *es* and *ei* characteristics, respectively) were cloned from human and rat tissues (29-32), and there is evidence from the human EST data base that a third ENT family member may exist (33). cDNAs encoding representatives of three CNT subfamilies (CNT1, CNT2 and CNT3: exhibit *cit*, *cif* and *cib* characteristics, respectively) were cloned from human, rat, pig and/or mouse tissues (28, 32, 34-39).

The ENT and CNT protein families do not share sequence identities and are architecturally quite different (Fig. 5). The ENT proteins are predicted to have 11 transmembrane domains (29, 30, 32, 33) whereas the CNT proteins of mammalian cells are predicted to have 13 transmembrane domains (40, 41). Studies with N-glycosylation mutants and antibody preparations raised against the large hydrophilic domains of hENT1 have established that the large hydrophilic loop between transmembrane domains 1 and 2 is extracellular and the large hydrophilic loop between transmembrane domains 6 and 7 is intracellular.

There has been a rapid increase in the identification of nucleoside transporter cDNAs since the isolation of the first cDNAs encoding representative members of the CNT and ENT families. Cloning strategies based on sequence similarities and functional analysis of recombinant proteins have recognized in excess of 20 nucleoside transporter cDNAs from eukaryotes and prokaryotes. Additionally, homology searches of sequence databases using predicted protein and/or cDNA sequences have identified a number of structurally related proteins that are candidate nucleoside transporters. The variability in the properties of the ENTs and CNTs among different cells and species has expanded beyond the scope of the existing nomenclature. Currently, nucleoside transporter proteins are categorized on the basis of their structural similarities as either CNTs or ENTs; their molecular and functional properties must be determined experimentally. Phylogenetic trees of known and putative nucleoside transporter proteins have been developed (33, 40, 42) and are constantly changing.



**Figure 5.** The architecture of the ENT and CNT protein families: different predicted membrane topologies

### **Therapeutic relevance: nucleoside transport activity is required for cytotoxicity of nucleoside drugs.**

Our studies of nucleoside transport in transplantable murine tumors and cultured murine and human cell lines led to the concept that cells require membrane ‘carriers’ for salvage of extracellular nucleoside precursors of nucleic acids, and for manifestation of cytotoxicity by anticancer nucleoside drugs. We demonstrated that inhibition of nucleoside transport by NBMPR afforded protection of cells against cytarabine, an anticancer nucleoside, by reducing drug uptake (43). This work, which was conducted with a mouse leukemia cell line (L1210), suggested that nucleoside transport activity is a critical determinant of cytotoxicity of nucleoside drugs. The requirement for nucleoside transport for cytotoxicity was extended to human cells in a subsequent study in which we demonstrated that treatment with non-toxic levels of NBMPR protected cultured human cells (RPMI 6410) from a large series of structurally diverse cytotoxic nucleosides by inhibiting cellular uptake of drug (13). We also demonstrated that resistance to nucleoside drugs in a murine lymphoma cell line that had been mutagenized and selected for resistance to cytotoxic nucleosides was due to the loss of a functional nucleoside transporter in cell membranes (21).

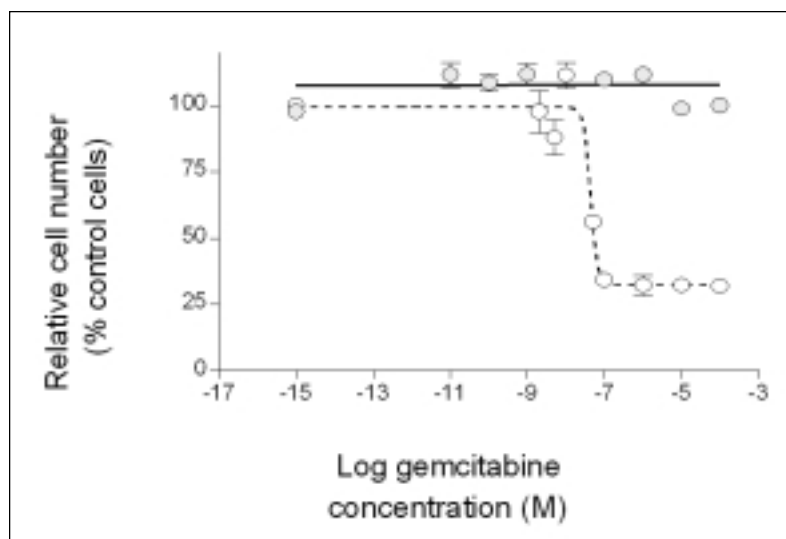
Our results established that either pharmacologic or genetic nucleoside-transport deficiency is accompanied by high-level resistance to a variety of nucleoside drugs and thus provided

experimental evidence for the concept that the presence of a functional nucleoside transporter in cell membranes is essential for manifestation of cytotoxicity of many nucleoside drugs. In recent studies with gemcitabine, we have shown that a panel of mutant murine leukemia L1210 cell lines that differ in their nucleoside transporter phenotypes exhibit different levels of gemcitabine sensitivity (Table 1).

**Table 1. Gemcitabine Cytotoxicity in Murine L1210 Leukemia Cells with Defined NT Activities**

Cell line	Origin	es	ei	cit	cif	IC <sub>50</sub> (μM)	Relative resistance
L1210	wild-type	+	+		+	0.0071	1.0
B23.1	mutant	+				0.092	13
DU-5	transfectant			+		0.012	1.7
MA27	mutant				+	5.2	730
DNC-3	null mutant					13	1800

We have also demonstrated that normal human hematopoietic progenitor cells can be protected from *ex vivo* nucleoside cytotoxicity by treatment with nucleoside transport inhibitors, thereby providing experimental evidence for a therapeutic rationale based on the use of nucleoside transport



**Figure 6.** Gemcitabine toxicity against NT-deficient (CEM-AraC-8C) and NT-competent (CEM) cells

inhibitors to selectively protect dose-limiting normal tissues during nucleoside chemotherapy (44). Our most recent studies (26) have shown a strong correlation between nucleoside transport activity and cytotoxicity against human cancer cell lines by gemcitabine, a widely used nucleoside drug with therapeutic activity against a variety of solid tumors (e.g., breast, pancreas, head and neck, lung). Fig. 6 compares the cytotoxicity of gemcitabine against cultured human leukemia cells that possess the capacity for nucleoside transport (CEM cells) with that against a mutant cell line (CEM-AraC-8C) that lacks the capacity for nucleoside transport.

### Future studies

We have proposed that cellular resistance to anticancer nucleoside drugs is related, in part, to low nucleoside transport capacity (45). That several anti-cancer cytidine analogs are transported differently by recombinant human nucleoside transporters (46, 47) is consistent with the importance of drug transportability as a determinant of therapeutic activity. We are collaborating with Dr. J.R. Mackey (Oncology, University of Alberta) on the role of nucleoside transporters in clinical resistance to gemcitabine therapy in solid tumors, with a special focus on cancers of the breast, head and neck and pancreas, and resistance to nucleoside therapy in hematologic malignancies. On-going and future projects include development of immunohistochemical assays for detection of nucleoside transporters in clinical samples (48), assessment of relationships between expression of nucleoside-transporter mRNA by cells and responsiveness to gemcitabine, and definition of the nucleoside-transporter phenotype of hematopoietic progenitor cells.

A major goal of our translational research program is to define nucleoside structural determinants for permeants and inhibitors of the human transporters, to guide the development and use of therapeutic nucleosides. We have taken advantage of our ability to functionally produce the human nucleoside transporters in yeast (49, 50) to develop assays to assess the potential transportability of nucleoside drugs by assessing the ability of test nucleosides to inhibit uptake of <sup>3</sup>H-uridine (a universally accepted permeant of the known human transporters). The determination of relationships between nucleoside transportability and cytotoxicity will be undertaken in human cell lines, since there are substantial differences in sensitivity to cytotoxic drugs between yeast and human cells. We

have created a series of stable transfectants each of which possesses a different human transporter subtype in otherwise genetically identical human cell lines. The transportability and inhibitor-sensitivity assays in yeast and transportability-cytotoxicity assays in human cell lines are being used to develop 'transportability guidelines' for development and application of therapeutic nucleosides for use in human diseases.

#### ACKNOWLEDGEMENTS:

*Our research is supported by operating grants from the National Cancer Institute of Canada, the Canadian Institutes of Health Research and the Alberta Cancer Board, by an award to the author from the Canada Research Chairs Program, by awards to research trainees from the Alberta Heritage Foundation for Medical Research, by infrastructure funding from the Alberta Cancer Board, the Alberta Cancer Foundation, the Canadian Foundation for Innovation and the Alberta Science and Research Authority. I am grateful to my long-time collaborators, S.A. Baldwin and J.D. Young, for their outstanding contributions to the nucleoside transport field and their generosity in sharing knowledge and reagents with members of my research group.*

#### References

1. Oliver, J. M. and Paterson, A. R. Nucleoside transport. I. A mediated process in human erythrocytes. *Canadian Journal of Biochemistry*. *49*: 262-70, 1971.
2. Paterson, A. R. and Oliver, J. M. Nucleoside transport. II. Inhibition by p-nitrobenzylthioguanosine and related compounds. *Canadian Journal of Biochemistry*. *49*: 271-4, 1971.
3. Cass, C. E., Gaudette, L. A., and Paterson, A. R. 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, 1974.
4. Cass, C. E. and Paterson, A. R. Inhibition by nitrobenzylthioinosine of uptake of adenosine, 2'-deoxyadenosine and 9-beta-D-arabinofuranosyladenine by human and mouse erythrocytes. *Biochem Pharmacol*. *24*: 1989-93, 1975.
5. Cass, C. E. and Paterson, A. R. Nitrobenzylthioinosine binding sites in the erythrocyte membrane. *Biochim Biophys Acta*. *419*: 285-94, 1976.
6. Cass, C. E. and Paterson, A. R. Mediated transport of nucleosides in human erythrocytes. Accelerative exchange diffusion of uridine and thymidine and specificity toward pyrimidine nucleosides as permeants. *J Biol Chem*. *247*: 3314-20, 1972.
7. Cass, C. E. and Paterson, A. R. Mediated transport of nucleosides by human erythrocytes. Specificity toward purine nucleosides as permeants. *Biochim Biophys Acta*. *291*: 734-46, 1973.
8. Cass, C. E., Belt, J. A., and Paterson, A. R. Adenosine transport in cultured cells and erythrocytes. *Prog Clin Biol Res*. *230*: 13-40, 1987.
9. Harley, E. R., Paterson, A. R., and Cass, C. E. Initial rate kinetics of the transport of adenosine and 4-amino-7-(beta-D-ribofuranosyl)pyrrolo[2,3-d] pyrimidine (tubercidin) in cultured cells. *Cancer Res*. *42*: 1289-95, 1982.
10. Paterson, A. R., Harley, E. R., and Cass, C. E. Inward fluxes of adenosine in erythrocytes and cultured cells measured by a quenched-flow method. *Biochem J*. *224*: 1001-8, 1984.
11. Paterson, A. R., Kolassa, N., and Cass, C. E. Transport of nucleoside drugs in animal cells. *Pharmacol Ther*. *12*: 515-36, 1981.
12. Paterson, A. R. P., Harley, E. R., Kolassa, N., and Cass, C. E. Transport of nucleosides in animal cells. *Nucleosides and Cancer Treatment*, pp. 3-17: Academic Press Australia, 1981.
13. Paterson, A. R., Yang, S. E., Lau, E. Y., and Cass, C. E. Low specificity of the nucleoside transport mechanism of RPMI 6410 cells. *Mol Pharmacol*. *16*: 900-8, 1979.
14. Paterson, A. R., Babb, L. R., Paran, J. H., and Cass, C. E. Inhibition by nitrobenzylthioinosine of adenosine uptake by asynchronous HeLa cells. *Mol Pharmacol*. *13*: 1147-58, 1977.
15. Cass, C. E. and Paterson, A. R. Inhibition of thymidine uptake in asynchronous HeLa cells by nitrobenzylthioinosine. *Exp Cell Res*. *105*: 427-35, 1977.
16. Paterson, A. R., Naik, S. R., and Cass, C. E. Inhibition of uridine uptake in HeLa cells by nitrobenzylthioinosine and related compounds. *Mol Pharmacol*. *13*: 1014-23, 1977.
17. Paterson, A. R. P., Jakobs, E. S., Harley, E. R., Fu, N., Robins, M. J., and Cass, C. E. Inhibition of nucleoside transport. *In*: R. M. Berne, T. W. Rall, and R. Rubio (eds.), *Regulatory function of adenosine*, pp. 203-220. Boston: M. Nijhoff Publishers, 1983.
18. Paterson, A. R. P., Jakobs, E. S., Harley, E. R., Cass, C. E., and Robins, M. J. Inhibitors of nucleoside transport as probes and drugs. *In*: Y. C. Cheng (ed.) *Development of target-oriented anticancer drugs*, pp. 41-55. New York, 1983.
19. Paterson, A. R. P., R., H. E., and Cass, C. E. Measurement and inhibition of membrane transport of adenosine. *In*: D. M. Paton (ed.) *Methods in Pharmacology*, Vol. 6, pp. 165-179: Plenum Publishing, 1985.
20. Dahlig-Harley, E., Eilam, Y., Paterson, A. R., and Cass, C. E. Binding of nitrobenzylthioinosine to high-affinity sites on the nucleoside-transport mechanism of HeLa cells. *Biochem J*. *200*: 295-305, 1981.
21. Cass, C. E., Kolassa, N., Uehara, Y., Dahlig-Harley, E., Harley, E. R., and Paterson, A. R. Absence of binding sites for the transport inhibitor nitrobenzylthioinosine on nucleoside transport-deficient mouse lymphoma cells. *Biochim Biophys Acta*. *649*: 769-77, 1981.
22. Koren, R., Cass, C. E., and Paterson, A. R. The kinetics of dissociation of the inhibitor of nucleoside trans-

- port, nitrobenzylthioinosine, from the high-affinity binding sites of cultured hamster cells. *Biochem J.* 216: 299-308, 1983.
23. Cass, C. E., Gati, W. P., Odegard, R., and Paterson, A. R. The effect of pH on interaction of nitrobenzylthioinosine and hydroxynitrobenzylthioinosine with the nucleoside transporter of human erythrocyte membranes. *Mol Pharmacol.* 27: 662-5, 1985.
  24. Paterson, A. R., Lau, E. Y., Dahlig, E., and Cass, C. E. A common basis for inhibition of nucleoside transport by dipyrnidamole and nitrobenzylthioinosine? *Mol Pharmacol.* 18: 40-4, 1980.
  25. Cass, C. E. Nucleoside Transport. In: N. H. Georgopapadakou (ed.) *Drug Transport in Antimicrobial and Anticancer Chemotherapy*, pp. 403-451. New York, NY: Marcel Dekker, 1995.
  26. Mackey, J. R., Mani, R. S., Selner, M., Mowles, D., Young, J. D., Belt, J. A., Crawford, C. R., and Cass, C. E. Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. *Cancer Res.* 58: 4349-57, 1998.
  27. Kwong, F. Y., Davies, A., Tse, C. M., Young, J. D., Henderson, P. J., and Baldwin, S. A. Purification of the human erythrocyte nucleoside transporter by immunoaffinity chromatography. *Biochem J.* 255: 243-9, 1988.
  28. Huang, Q. Q., Yao, S. Y., Ritzel, M. W., Paterson, A. R., Cass, C. E., and Young, J. D. Cloning and functional expression of a complementary DNA encoding a mammalian nucleoside transport protein. *J Biol Chem.* 269: 17757-60, 1994.
  29. Griffiths, M., Beaumont, N., Yao, S. Y., Sundaram, M., Boumah, C. E., Davies, A., Kwong, F. Y., Coe, I., Cass, C. E., Young, J. D., and Baldwin, S. A. Cloning of a human nucleoside transporter implicated in the cellular uptake of adenosine and chemotherapeutic drugs. *Nat Med.* 3: 89-93, 1997.
  30. Griffiths, M., Yao, S. Y., Abidi, F., Phillips, S. E., Cass, C. E., Young, J. D., and Baldwin, S. A. Molecular cloning and characterization of a nitrobenzylthioinosine-insensitive (ei) equilibrative nucleoside transporter from human placenta. *Biochem J.* 328: 739-43, 1997.
  31. Crawford, C. R., Patel, D. H., Naeve, C., and Belt, J. A. Cloning of the human equilibrative, nitrobenzylmercaptapurine riboside (NBMPR)-insensitive nucleoside transporter ei by functional expression in a transport-deficient cell line. *J Biol Chem.* 273: 5288-93, 1998.
  32. Yao, S. Y., Ng, A. M., Muzyka, W. R., Griffiths, M., Cass, C. E., Baldwin, S. A., and Young, J. D. Molecular cloning and functional characterization of nitrobenzylthioinosine (NBMPR)-sensitive (es) and NBMPR-insensitive (ei) equilibrative nucleoside transporter proteins (rENT1 and rENT2) from rat tissues. *J Biol Chem.* 272: 28423-30, 1997.
  33. Hyde, R. J., Cass, C. E., Young, J. D., and Baldwin, S. A. The ENT family of eukaryote nucleoside transporters: recent advances in the investigation of structure/function relationships and the identification of novel isoforms. *Mol Membrane Biol.* 18: 53-63, 2001.
  34. Yao, S. Y., Ng, A. M., Ritzel, M. W., Gati, W. P., Cass, C. E., and Young, J. D. Transport of adenosine by recombinant purine- and pyrimidine-selective sodium/nucleoside cotransporters from rat jejunum expressed in *Xenopus laevis* oocytes. *Mol Pharmacol.* 50: 1529-35, 1996.
  35. Ritzel, M. W., Yao, S. Y., Huang, M. Y., Elliott, J. F., Cass, C. E., and Young, J. D. Molecular cloning and functional expression of cDNAs encoding a human Na<sup>+</sup>-nucleoside cotransporter (hCNT1). *Am J Physiol.* 272: C707-14, 1997.
  36. Wang, J., Su, S. F., Dresser, M. J., Schaner, M. E., Washington, C. B., and Giacomini, K. M. Na(+)-dependent purine nucleoside transporter from human kidney: cloning and functional characterization. *Am J Physiol.* 273: F1058-65, 1997.
  37. Pajor, A. M. Sequence of a pyrimidine-selective Na<sup>+</sup>/nucleoside cotransporter from pig kidney, pkCNT1. *Biochim Biophys Acta.* 1415: 266-9, 1998.
  38. Patel, D. H., Crawford, C. R., Naeve, C. W., and Belt, J. A. Cloning, genomic organization and chromosomal localization of the gene encoding the murine sodium-dependent, purine-selective, concentrative nucleoside transporter (CNT2) [In Process Citation]. *Gene.* 242: 51-8, 2000.
  39. Ritzel, M. W. L., Ng, A. M. L., Yao, S. Y. M., Graham, K., Loewen, S. K., Smith, K. M., Ritzel, R. G., Mowles, D. A., Carpenter, P., Chen, X.-Z., Karpinski, E., Hyde, R. J., Baldwin, S. A., Cass, C. E., and Young, J. D. Molecular identification and characterization of novel human and mouse concentrative Na<sup>+</sup>-nucleoside cotransporter proteins (hCNT3 and mCNT3) broadly selective for purine and pyrimidine nucleosides (system *cib*). *J Biol Chem.* 276: 2914-2927, 2001.
  40. Ritzel, M. W. L., Ng, A. M. L., Yao, S. Y. M., Graham, K., Loewen, S. K., Smith, K. M., Hyde, R. J., Karpinski, E., Cass, C. E., Baldwin, S. A., and Young, J. D. Recent molecular advances in studies of the concentrative Na<sup>+</sup>-dependent nucleoside transporter (CNT) family: Identification and characterization of novel human and mouse proteins (hCNT3 and mCNT3) broadly selective purine and pyrimidine nucleosides (Systems *cib*). *Mol Membrane Biol.* 18: 65-72, 2001.
  41. Hamilton, S. R., Yao, S. Y. M., Ingram, J. C., Hadden, D. A., Ritzel, M. W. L., Gallagher, M. P., Henderson, P. J. F., Cass, C. E., Young, J. D., and Baldwin, S. A. Sub-cellular distribution and membrane topology of the rat concentrative Na<sup>+</sup>-nucleoside co-transporter rCNT1. *J Biol Chem.* 276: 27981-27988, 2001.
  42. Vickers, M. F., Young, J. D., Baldwin, S. A., Mackey, J. R., and Cass, C. E. Nucleoside transporter proteins: emerging targets for drug discovery. *Emerging Therapeutic Targets.* 4: 515-539, 2000.
  43. Cass, C. E., Muzik, H., and Paterson, A. R. Combination therapy of mouse leukemia L1210 by 1-beta-D-arabinofuranosylcytosine and 6-[(4-nitrobenzylthio)-9-beta-D-ribofuranosyl]purine. *Cancer Res.* 35: 1187-93, 1975.
  44. Cass, C. E., King, K. M., Montano, J. T., and Janowska-Wieczorek, A. A comparison of the abilities of nitrobenzylthioinosine, dilazep, and dipyrnidamole to protect human hematopoietic cells from 7-

- deazaadenosine (tubercidin). *Cancer Res.* 52: 5879-86, 1992.
45. Mackey, J. R., Baldwin, S. A., Young, J. D., and Cass, C. E. Nucleoside transport and its significance for anticancer drug resistance. *Drug Resistance Updates.* 1: 310-324, 1998.
46. Mackey, J. R., Yao, S. Y., Smith, K. M., Karpinski, E., Baldwin, S. A., Cass, C. E., and Young, J. D. Gemcitabine transport in xenopus oocytes expressing recombinant plasma membrane mammalian nucleoside transporters. *J Natl Cancer Inst.* 91: 1876-81, 1999.
47. Graham, K. A., Leithoff, J., Coe, I. R., Mowles, D. A., Mackey, J. R., Young, J. D., and Cass, C. E. Differential transport of cytosine-containing nucleosides by recombinant concentrative nucleoside transporter protein hCNT1. *Nucleosides and Nucleotides.* 19: 415-434, 2000.
48. Mackey, J. R., Jennings, L. L., Clarke, M. L., Santos, C. L., Dabbagh, L., Vsianska, M., Koski, S. L., Coupland, R. W., Baldwin, S. A., Young, J. D., and Cass, C. E. Immunohistochemical variation of human equilibrative nucleoside transporter 1 (hENT1) protein in primary breast cancers. *Clinical Cancer Research*, in press, 2001.
49. Vickers, M. F., Yao, S. Y. M., Baldwin, S. A., Young, J. D., and Cass, C. E. Nucleoside transporter proteins of *Saccharomyces cerevisiae*: demonstration of a transporter (FUI1) with high uridine selectivity in plasma membranes and a transporter (FUN26) with broad nucleoside selectivity in intracellular membranes. *J. Biol. Chem.* 275: 25931-25938, 2000.
50. Vickers, M. F., Mani, R. S., Sundaram, M., Hogue, D. L., Young, J. D., Baldwin, S. A., and Cass, C. E. Functional production and reconstitution of the human equilibrative nucleoside transporter (hENT1) in *Saccharomyces cerevisiae*. Interaction of inhibitors of nucleoside transport with recombinant hENT1 and a glycosylation-defective derivative (hENT1/N48Q). *Biochem J.* 339: 21-32, 1999.