The mode of transport of a nonphosphorylated adenosine analog, 5'-deoxyadenosine, was studied in murine leukemia L1210 cells. This compound is not subject to the action of intracellular nucleoside-trapping kinases, and its transport can be examined without regard for effects of experimental conditions on kinase activity. Accumulation of 5'-deoxyadenosine was rapid, and nonconcentrative, with equilibrium attained within 12 s at 37°C. Kinetic studies were carried out at 20°C. We found both a nonmediated (diffusion) and a mediated transport process. The latter had an apparent $K_m$ of 115 μM, $V_{max} = 105$ pmol/10^6 cells/min. Uptake of 5'-deoxyadenosine was inhibited by several heterologous nucleosides including adenosine, 2'-deoxyadenosine, thymine riboside, and inosine. Like 2'-deoxyadenosine, 5'-deoxyadenosine was more lipid-soluble than adenosine (from octanol/water partition studies). Compared with 5'-deoxyadenosine, adenosine had a much lower apparent $K_m$ (5 μM) and a higher $Q_{10}$ over the 27-37°C range (3.0 versus 1.3). Data obtained with adenosine might, however, reflect properties of intracellular adenosine kinase interacting with a transport process.

Transport of nucleosides across a cell membrane is often measured by determining the rate of incorporation of an endogenously supplied radioactive compound into nondiffusible intracellular material representing nucleotides + nucleic acid (1-7). In cultured animal cells, it is generally assumed that the rate of transport, rather than of intracellular phosphorylation, is rate-limiting (1). Recent evidence has suggested that the apparent kinetics of certain transport processes are affected by properties of nucleoside kinases (8, 9). Furthermore, effects of different drugs on the nucleoside kinases might be taken to represent alteration of transport processes unless appropriate methods for delineation of transport versus phosphorylation were available.

The use of low temperatures to minimize kinase activity might permit measurement of kinetics of transport alone, but many kinases are functional even near 0°C (10-12). The use of

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Experimental Procedures

Materials

Radioactive 5'-deoxyadenosine, labeled with tritium at the 5' position, was prepared by New England Nuclear Corp., Boston, as follows. One hundred milligrams of 5'-ido-5'-deoxyadenosine (Aldrich Chemical Co., Milwaukee) was dissolved in 2 ml of water, then 250 μg of ethylamine and 150 mg of 5% Pd/Al₂O₃ were added. This mixture was stirred overnight in the dark with 25 μCi of tritium. Labile tritium was removed by lyophilization, and the product was delivered to us. Using Solvent A, l-propanol:CHCl₃ (99:1), and a TLC (silica) system, we found it to be 85% pure. Authentic samples of 5'-deoxyadenosine provided by Research Plus Laboratories, Denver, N. J., and by ICN Pharmaceuticals, Cleveland, were used to characterize the behavior of the nucleoside ($R_f = 0.4$) in this system. The starting material, 5'-ido-5'-deoxyadenosine exhibited an $R_f$ value of 0.5. The purified radioactive product was eluted from the silica sheet, diluted with carrier to a specific activity of 6 x 10⁶ dpm/pmol, and stored in 50% ethanol at -70°C. The product was found to be chromatographically pure in another TLC (cellulose) system using Solvent B, saturated ammonium sulfate:0.1 M phosphate buffer, pH 6.8:1-propanol (100:60:2).

1°C)Adenosine and [14C]deoxyadenosine were purchased from New England Nuclear and were purified on TLC (silica) as described above. Nonradioactive nucleosides were purchased from Sigma Chemical Co., St. Louis, and from Calbiochem Corp., Los Angeles. Persantin (dipyridamole) was provided by Geigy Pharmaceuticals, Yonkers, N. Y., and deuteroporphyrin IX (NSC 19663) by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

Methods

Cell Culture—Murine leukemia L1210 cells were grown in tissue culture using minimal essential Eagle's medium (spinner) supplemented with 10% fetal calf serum, double the normal level of glutamine, and gentamycin. The cells were collected during the latter portion of the exponential growth curve, and suspended in a modified growth medium in which Heps¹ buffer, pH 7.4, replaced NaHCO₃. This substitution permitted short term incubations at kinase-less mutants (8, 9) or of cells depleted of ATP pools (13) are other alternatives. But, for the study of nucleoside transport in a variety of different wild type cell lines, under physiological conditions, a nonphosphorylated nucleoside would be desirable. Such a compound would be analogous to the nonmetabolized amino acid model, cycloleucine (14). In this report, we describe such a nucleoside, 5'-deoxyadenosine, which is readily prepared in radioactive form, and is not phosphorylated by at least two widely studied mammalian cell lines, the murine L1210 lymphocytic leukemia and the Ehrlich ascites tumor cell.

* Transport of a Nonphosphorylated Nucleoside, 5'-Deoxyadenosine, by Murine Leukemia L1210 Cells*

(Received for publication, June 23, 1977)
Results

Properties of Nucleosides—The purified preparation of radioactive 5'-deoxyadenosine was stable in 50% ethanol at −70°C for at least 3 months. An initial radiopurity level of 98% had fallen to 95% over this interval. The major impurity apparently represents the result of a reaction liberating a fragment from the nucleoside molecule; a brief treatment of radioactive 5'-deoxyadenosine with 1 M HCl produced a labeled product with the same R_f as the major impurity described above.

The water:octanol partition ratios of 5'-deoxyadenosine was 4.02:1. A similar value (5.14:1) was obtained with 2'-deoxy-5'-deoxyadenosine with 1 M HCl produced a labeled product likely represents the result of a reaction liberating a fragment from the nucleoside molecule; a brief treatment of radioactive 5'-deoxyadenosine with 1 M HCl produced a labeled product with the same R_f as the major impurity described above.

We found evidence for neither phosphorylation (Table I) nor deamination of 5'-deoxyadenosine, but adenosine was more hydrophilic, with a value of 13.1.1.

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\[ \text{Table I} \]

<table>
<thead>
<tr>
<th>Nucleoside</th>
<th>Kinase activity (fmol/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uridine</td>
<td>4.6</td>
</tr>
<tr>
<td>Deoxyctydine</td>
<td>9.3</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7.0</td>
</tr>
<tr>
<td>2'-Deoxyadenosine</td>
<td>5.1</td>
</tr>
<tr>
<td>5'-Deoxyadenosine</td>
<td>&lt;0.15</td>
</tr>
</tbody>
</table>

Inhibition of Accumulation—We tested KCN, persantin, HgCl2/NaI, and photo-activated deuteroporphyrin IX. The latter was found (25) to cause a substantial inhibition of the accumulation of nucleosides by L1210 cells (see "Discussion").
5'-Deoxyadenosine Transport

Temperature (°C)

![Figure 1](image)

Fig. 1. Kinetics of uptake of 5'-deoxyadenosine (A) and of adenosine (B) as a function of incubation temperature. Initial rates were measured as described in the text.

![Figure 2](image)

Fig. 2. Plot of rate of uptake of 5'-deoxyadenosine as a function of the extracellular concentration, at 20°C, measured during 18-s incubations. The mediated (M) and diffusion (D) components of transport were calculated as described in Ref. 20.

The data of Table II were obtained using 10 μM nucleoside for 18 s at 20°C. The solution of HgCl₂/NaI (in isotonic NaCl) essentially abolished accumulation of adenosine, 2'-deoxyadenosine and 5'-deoxyadenosine. The porphyrin and persantin were less effective inhibitors of uptake of the latter two agents, perhaps reflecting the greater diffusion component of their uptake. In other studies, we found that a 5 to 10 mM level of KCN failed to affect accumulation of 5'-deoxyadenosine at any nucleoside level tested.

Competition Studies—Addition of a 250-fold excess of non-

![Figure 3](image)

Fig. 3. A, kinetics of 5'-deoxyadenosine transport, calculated from the mediated component of transport (see Fig. 2). B, rate of efflux, as a function of temperature, of 5'-deoxyadenosine from cells preloaded with 150 mM of the nucleoside. The loading incubation was carried out at 10°C over a 3-min interval.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Adenosine</th>
<th>2'-Deoxyadenosine</th>
<th>5'-Deoxyadenosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persantin</td>
<td>7</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>HgCl₂/NaI</td>
<td>2</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Porphyrin</td>
<td>8</td>
<td>11</td>
<td>18</td>
</tr>
</tbody>
</table>

Table II

Inhibition of nucleoside uptake

L1210 cells were incubated for 18 s at 20°C with 10 μM persantin or 2 mM HgCl₂ + 1.2 mM NaI (in isotonic NaCl) + 10 μM labeled nucleoside; the cells were then collected by centrifugation, and accumulation of labeled substrate measured. Cells were incubated with 10 μg/ml of deuteroporphyrin IX in light (24), then suspended in fresh medium and incubated for 18 s at 20°C with labeled nucleoside as described above. Data are shown in terms of percentage of control (untreated) values.

<table>
<thead>
<tr>
<th>Added nucleoside</th>
<th>5'-Deoxyadenosine accumulation</th>
<th>Relative affinity of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>16</td>
<td>2.14</td>
</tr>
<tr>
<td>2'-Deoxyadenosine</td>
<td>20</td>
<td>1.63</td>
</tr>
<tr>
<td>5'-Deoxyadenosine</td>
<td>29</td>
<td>1.00</td>
</tr>
<tr>
<td>Thymine ribose</td>
<td>30</td>
<td>0.95</td>
</tr>
<tr>
<td>Thymidine</td>
<td>48</td>
<td>0.44</td>
</tr>
<tr>
<td>Inosine</td>
<td>34</td>
<td>0.79</td>
</tr>
<tr>
<td>2'-Deoxyinosine</td>
<td>47</td>
<td>0.46</td>
</tr>
<tr>
<td>Guanosine</td>
<td>41</td>
<td>0.59</td>
</tr>
<tr>
<td>2'-Deoxyguanosine</td>
<td>51</td>
<td>0.39</td>
</tr>
<tr>
<td>Uridine</td>
<td>66</td>
<td>0.21</td>
</tr>
<tr>
<td>2'-Deoxyuridine</td>
<td>51</td>
<td>0.39</td>
</tr>
<tr>
<td>Cytidine</td>
<td>72</td>
<td>0.16</td>
</tr>
<tr>
<td>2'-Deoxycytidine</td>
<td>61</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table III

Inhibition of 5'-deoxyadenosine transport by heterologous nucleosides

L1210 cells were incubated with 1 μM 5'-deoxy[¹³C]adenosine alone, or in the presence of 250 μM levels of other nucleosides, for 18 s at 20°C. Transport was then stopped with the HgCl₂/NaI solution as described under "Methods," and accumulation of radioactive material by the cells was measured. Relative inhibition of uptake by nonradioactive compounds is shown, along with the relative affinity of competing nucleosides for transport calculated as described in Ref. 27.
radioactive nucleosides inhibited accumulation of labeled 5'-deoxyadenosine (Table III). The most potent inhibitors were adenosine, 2'-deoxyadenosine, nonradioactive 5'-deoxyadenosine, thymine riboside, inosine, and guanosine, in that order. Except for uridine and cytidine, we found the ribose derivatives to be more effective inhibitors of 5'-deoxyadenosine transport than were the 2'-deoxyribose derivatives.

**Exodus of 5'-deoxyadenosine**—Cells were preloaded with 150 μM of labeled compound by a 10-min incubation at 0°, and were then suspended in fresh medium at 0°, 15°, or 37°. The rate of loss of label from the cells was measured as described under "Methods." The data show a substantial temperature dependence of nucleoside exodus, Fig. 3B.

**DISCUSSION**

This study described studies of transport of a radioactive nonmetabolized nucleoside which is not affected by the properties of intracellular nucleoside-trapping reactions, e.g., kinases. Previous reports had indicated that the kinetics of thymidine transport in cultured animal cells were altered when thymidine kinase activity was inhibited (8, 9). Since the levels of activity of nucleoside kinases are potent determinants of nucleoside accumulation, it is not always easy to distinguish effects of external agents on mediated transport versus kinase activity, and careful experiments need to be planned so that distinctions can be made, e.g. Refs. 3-5, 26.

Once a purified preparation of 6'-deoxyadenosine was obtained, we determined that this agent was not significantly phosphorylated by our model system, the transplantable murine leukemia L1210 cell line. In a recent report, the compound was also found to be neither phosphorylated nor deaminated by Ehrlich ascites tumor cells (28). Uptake of 5'-deoxyadenosine was found to be nonconcentrative; in the absence of an appropriate kinase, equilibration between external and internal nucleoside pools was very rapid. To slow the rate of uptake sufficiently to permit valid rate measurements over 6- to 18-s intervals, our experiments were mainly done at 20°.

In contrast to the markedly temperature-sensitive uptake of adenosine (Fig. 1), 5'-deoxyadenosine uptake was much less affected by incubation temperature; furthermore, the rate of uptake was almost a linear function of temperature over the 0-37° range (Q10 = 1.3). The Q10 for adenosine, over 27-37° was approximately 3.0. These data suggest that the apparent temperature dependence of adenosine transport may be approximately twice that of 2'-deoxyadenosine. This difference could reflect the effect of elimination of intracellular kinase as a factor in nucleoside accumulation, analogous to the finding of an elevated Km for thymidine transport in kinase-less or ATP-depleted cells (8, 13). However, another possibility is impaired affinity of 5'-deoxyadenosine for a transport carrier protein. Our studies of apparent inhibition constants (Table III) suggest this latter possibility.

Both adenosine and 2'-deoxyadenosine were more potent inhibitors of transport of labeled 5'-deoxyadenosine than was nonradioactive 5'-deoxyadenosine. Inosine and thymine riboside were also effective inhibitors of 5'-deoxyadenosine transport. In another report (24), Plagemann found inosine, uridine, and thymidine were the most potent nucleoside inhibitors of adenosine transport, while 2'-deoxyadenosine uptake was most effectively inhibited by deoxyinosine, adenosine, and guanosine (25). These data suggest that 5'-deoxyadenosine may enter the cell via several access systems, mainly that normally utilized by adenosine.

We found that uptake of 5'-deoxyadenosine was not inhibited by KCN, and was inhibited by persantin and a membrane-disruptive porphyrin (26), but to a lesser extent than either 2'-deoxyadenosine and adenosine. The data of Table II suggest that these two inhibitors are most effective against adenosine, a compound with only a minor capacity to diffuse into cells at low external concentrations. The HpCl/NaI solution blocked transport of all three nucleosides, including exodus of 5'-deoxyadenosine.

We propose 5'-deoxyadenosine as a nonmetabolized nucleoside analog for the study of nucleoside transport, and the effect thereon of external agents. The only drawbacks appear to be the need for rapid sampling times or reduced temperature of incubation and the somewhat greater diffusion component of transport than is found for the ribonucleosides, e.g., adenosine.

**Acknowledgments**—I thank Dr. Joseph Cory, University of South Florida, School of Medicine, for helpful discussions, and Gwynne Smith for excellent technical assistance.

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Transport of a nonphosphorylated nucleoside, 5'-deoxyadenosine, by murine leukemia L1210 cells.

D Kessel


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