Membrane-associated Enzymes Involved in Nucleoside Processing by Plasma Membrane Vesicles Isolated from L<sub>929</sub> Cells Grown in Defined Medium

CHIEN-CHUNG LI$ AND JOY HOCHSTADT

From the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Transport-competent plasma membrane vesicles isolated from a subline of L<sub>929</sub> cells (L<sub>929ae</sub>) grown in serum-free, defined medium accumulate ribose-1-P when exposed to adenosine, inosine, guanosine, or uridine. This observation suggests the action of one or more nucleoside phosphorylases acting prior to, during, or subsequent to the transport event. Extravesicular ribose-1-P neither inhibits uptake nor exchanges with intravesicular ribose-1-P, indicating that the action of the phosphorylase is not prior to uptake. Preloading the vesicles with inosine prior to subjecting the vesicles to conditions under which further uptake could not take place (in the presence of caffeine) did not result in an alteration of the ribose-1-P to inosine ratio within the vesicles. This observation was interpreted as evidence that only exogenously derived, not intravesicular inosine, is the substrate for the nucleoside phosphorylase. This datum, when taken with the fact that hypoxanthine is never found to a significant extent within the vesicles, suggests that the phosphorolytic cleavage of inosine occurs as a group translocation during the transport event itself, so that hypoxanthine is released to the surrounding medium while the ribose 1-P accumulates intravesicularly. Thus, phosphorolysis would seem to occur during passage across the membrane.

Adenosine utilization by these plasma membrane vesicles appears to be closely linked to the presence of adenosine deaminase, although adenosine may also be taken up unchanged by intact cells. The relationship of other nucleic acid precursor-utilizing enzymes to transport and to the membrane has also been investigated. It was found that nucleoside phosphorylase, adenosine deaminase, and hypoxanthine phosphoribosyltransferase appear to co-purify with the vesicles. Adenosine kinase was not found to be associated with the isolated plasma membranes, nor was it involved with any transport process carried out by the plasma membrane vesicles.

Isolated plasma membrane preparations can be successfully used to study a variety of transport (1–5) and other problems in mammalian cells in culture (6, 7). Although techniques other than those leading to vesiculation, i.e. those resulting in membrane sheets (8, 9), have also been extremely useful in preparing highly purified membranes for certain structural characterizations, the closed vesicle appears to be a necessary prerequisite for the rapid and convenient measurement of transport across the membrane by currently available means of assay (10). For certain transport systems, vectorial metabolic processes which may directly result from action of enzymes associated with the membrane are essential to transport. In bacteria, the membrane transport systems for the utilization of sugars (11, 12) and purines (13–15) are examples of such mechanisms. In animal cells, the accumulation of the ribose moiety of inosine also appears to be an enzyme-mediated group translocation mechanism (4, 5). Membrane preparations used for studies of such systems also require, in addition to complete vesiculation, the preservation of the microenvironment of the membrane, especially the spatial arrangement between the components necessary for both transport and catalysis (cf. Ref. 16).

In the accompanying paper (17), we have identified two fractions of plasma membrane, each with characteristic physical and enzymatic properties; both are capable of transport. One of these two fractions, plasma membrane fraction II (PM II), was studied for its ability to take up radioactivity from nucleosides as a function of time, membrane and substrate concentration, pH, and concentration of a variety of inhibitors. We will now present results to show that the radioactivity accumulated in the case of each nucleoside is largely ribose-1-P, and that membrane-associated enzymes appear involved in the uptake process.
Enzymes Involved in Vesicle Transport

MATERIALS AND METHODS

Materials

[8-14C]adenosine (47mCi/mmol); [8-14C]adenine, (59 mCi/mmol); and [8-14C]hypoxanthine (69 mCi/mmol) were all purchased from Amer sham/Searle, Chicago, Ill. Other materials used have been detailed in the preceding paper (17) or in Ref. 10.

Methods other than those described in the preceding paper (17) are detailed in Ref. 10 or are as follows.

Cell Culture

The L105 cell line was purchased from Flow Laboratories (Rockville, Md.). Both A1 cells and its revertant cells (A1r) were obtained from Dr. O. Wesley McBride at the National Institutes of Health, Bethesda, Md. These cells were all propagated in spinner culture in Joklik modified Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum. Cell densities were maintained at 1 x 10^5 to 5 x 10^5 cells/ml. All cell cultures have been periodically tested for mycoplasma by microbiological culture (HEM Research, Baltimore, Md.).

Chromatographic Analysis of Transport Products in Intravesicular Spaces

Identification of Intravesicular Products—At the end of the incubation for the transport assay, the membrane vesicles were collected on 0.2-μm pore size nitrocellulose filters and extracted with 1 ml of 1 N NH4OH for 30 min at room temperature. The extracts were then lyophilized and dissolved in a small volume of distilled water. Aliquots of this solution were analyzed by thin layer chromatography at room temperature on plastic-backed cellulose sheets (Eastman, 6065). Identification of Products in Extravesicular Medium—The first 1 ml of filtrate was used directly for the analysis by chromatography on the cellulose thin layers.

The following three solvents were used for the chromatographic analysis: Solvent 2 has been described before (18). Solvents 9 and 27 consisted, respectively, of water-saturated (NH4)2SO4/0.1 M phosphate buffer, pH 6.0/isopropanol, 79/19/2; and isobutryric acid/1 N NH4OH/0.2 M EDTA, 100/60/8, and correspond to identically numbered solvents described in the Schwarz/Mann catalog. Appropriate authentic standards were cochromatographed with the samples so that spots corresponding to various compounds could be easily located under an ultraviolet lamp. Occasionally, chromatograms were further confirmed by radioautography with non-screen x-ray films (Kodak).

Enzyme Assays

All enzymes were assayed at 37°, in a total reaction mixture of 20 μl. For each assay, the enzyme and the basic buffer mixture were preincubated at 37° for 30 s before the addition of the appropriate [3H]-substrate to start the reaction. At the end of the incubation, which was usually 10 min, 40 μl of ice-cold 100% ethanol were added to terminate the reaction. Aliquots of the clear alcoholic supernatant were used for analysis by thin layer chromatography as described above. The amount of protein used in each assay was either 35 μg (when the homogenate was the enzyme source) or 29 μg (when the membrane preparation was the enzyme source).

The composition of the reaction mixtures are as follows: for purine-nucleoside phosphorylase (EC 2.4.2.1) and adenosine deaminase (EC 3.5.4.4); potassium phosphate, pH 7.5 (50 mM)/NaCl (100 mM)/[U-14C]inosine or adenosine, respectively (10 μM); for hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); Tris-HCl pH 7.5 (50 mM)/MgCl2 (5 mM)/phosphoribosylpyrophosphate (1 mM)/[8-14C]hypoxanthine (0.1 mM); for adenosine kinase (EC 2.7.1.20); Tris-HCl pH 7.0 (50 mM)/MgCl2 (4 mM)/ATP (10 mM)/[8-14C]adenosine (36 mM). Triton X-100, whenever used, was 0.2%.

Assay for Uptake of Nucleosides and Bases by Whole I-cells

Cells harvested from spinner or roller bottles were washed three times with cold Joklik modified MEM without serum and resuspended in the same medium to a final cell density of 0.5 x 10^6 cells/ml. Each assay tube contained 0.2 ml of the cell suspension, and 5 μl of 0.36 μM Tris-HCl, pH 7.4. After being equilibrated at 37° for 5 min, the reaction was started by the addition of radioactive substrate [8-14C]adenosine, [8-14C]adenine, or [8-14C]hypoxanthine, to a final concentration of 46 μM (usually 10 μl) unless otherwise indicated. At the end of the incubation, 1.5 ml of cold 0.8 M NaCl solution were added to terminate the reaction; filtration with suction on nitrocellulose filters (0.2 μm pore size) followed immediately. The filters were immediately washed with two 1.5-ml portions of the same solution, dried, and removed from the suction apparatus. They were then counted in a gas flow counter (Nuclear Chicago) at 18% efficiency. For each set of assays, the radioactivity trapped in the extracellular space was obtained by diluting the reaction mixture with 1.5 ml of cold 0.8 M NaCl solution before addition of the radioactive substrate and just before filtering as above; this value was subtracted from the experimental values obtained.

RESULTS

Chromatographic Identification of Vesicle Contents—Fig. 1 shows that the radioactivity accumulated in the vesicles after they are exposed to [U-14C]nucleotides in the medium is almost entirely ribose-1-P. The concentrations reached by each of the intravesicular products are shown in Table I, based on the determination of intravesicular volume of 2.8 μg/ml of protein (17). In this experiment, substrate concentrations were deliberately low to permit maximum concentration of intravesicular products. The finding that ribose-1-P was the major product indicates the involvement of nucleoside phosphoribosyltransferase(s) which mediate(s) the reaction:

Nucleoside + P1 = Ribose-1-P + Base

The appearance of inosine in the vesicles exposed to adenosine also indicates action of adenosine deaminase. Finally, the appearance of small amounts of AMP and IMP within the vesicles suggests both the presence of either phosphoribosyltransferases or nucleoside kinases and the presence of small stores of phosphoribosyl energy donors. The latter, though wholly unexpected, were also found in purified membranes from prokaryotes (11–13, 16).

Plasma Membrane Localization of Enzymes of Nucleic Acid Precursor Utilization—Table II shows that while none of the enzymes assayed appear to largely reside on the vesicles after membrane purification, the degree to which these enzyme activities do remain membrane-associated differs widely. Adenosine kinase appears to be entirely separated from the vesicles during membrane isolation, while the other three enzymes appear in the vesicle fractions. Further, enhancement of enzyme activity in the presence of 0.2% Triton is observed for the enzymes which tend to have some membrane association.

![Graph](http://example.com/graph.png)
Enzymes Involved in Vesicle Transport

TABLE I

Concentration of vesicular contents

Calculations are based on results presented in Fig. 1, taking the intravesicular volume as 2.8 μl/mg of membrane protein (cf. Ref. 17).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Time (min)</th>
<th>Ribose-1-P</th>
<th>Inosine</th>
<th>Adenosine</th>
<th>Guanosine</th>
<th>Uridine</th>
<th>IMP</th>
<th>AMP</th>
<th>GMP</th>
<th>UMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inosine (10 μM)</td>
<td>2</td>
<td>44.6</td>
<td>0</td>
<td>4.8</td>
<td>5.9</td>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>54.6</td>
<td>0</td>
<td>4.8</td>
<td>5.9</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>142</td>
<td>0</td>
<td>4.8</td>
<td>5.9</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>155</td>
<td>7.3</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenosine (10 μM)</td>
<td>2</td>
<td>15.7</td>
<td>7.3</td>
<td>5.9</td>
<td>0.4</td>
<td>0</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>41.2</td>
<td>0.9</td>
<td>5.9</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>80.3</td>
<td>3.5</td>
<td>2.6</td>
<td>3.8</td>
<td>8.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>155</td>
<td>7.3</td>
<td>0</td>
<td>4.8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanosine (8 μM)</td>
<td>2</td>
<td>19.8</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>23.9</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>36.8</td>
<td>0.3</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td></td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>48.8</td>
<td>0.6</td>
<td>0.3</td>
<td>3.9</td>
<td>3.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine (8.5 μM)</td>
<td>2</td>
<td>4.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>13.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>23.0</td>
<td>1.7</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>35.7</td>
<td>1.7</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE II

Relative specific activities of membrane-associated enzymes

Determination of enzyme activities is described under “Materials and Methods.” Relative specific activity is defined as the ratio of specific activity of the enzyme in the membrane vesicles to that in the homogenate.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Specific activity (nmoi/mg protein/min)</th>
<th>Specific activity in plasma membrane specific activity in homogenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase</td>
<td>Homogenate</td>
<td>1.46</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>PM*</td>
<td>0.88</td>
<td>1.27</td>
</tr>
<tr>
<td>Inosine phosphorylase</td>
<td>Homogenate</td>
<td>1.16</td>
<td>1.64</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>0.29</td>
<td>0.49</td>
</tr>
<tr>
<td>Hypoxanthine-guanine phosphoribosyl transferase</td>
<td>Homogenate</td>
<td>1.75</td>
<td>3.34</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>0.17</td>
<td>0.54</td>
</tr>
<tr>
<td>Adenosine kinase</td>
<td>Homogenate</td>
<td>1.46</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>PM</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*PM, plasma membrane vesicles.

Nucleoside Phosphorylase(s) and Vesicle Transport—Since ribose-1-P was always the predominant product accumulated in the vesicles exposed to uridine or purine nucleosides, we attempted to determine where it acted in the sequence of catalytic and transport events.

The first experiments were aimed at testing whether phosphorylase preceded uptake, and we were, in fact, measuring the transport of ribose-1-P. If there were a ribose-1-P transport system, internal ribose-1-P and external ribose-1-P would exchange across the membrane. Fig. 2 shows the data obtained when a number of compounds were evaluated in order to determine which ones might cause exchange or efflux across the membrane. In these experiments, the concentration of radioactive inosine used was about 6 times higher than usual, and the incubation time was prolonged to maximize the accumulation. Preloaded vesicles were then diluted 11-fold with the incubation buffer containing the test compounds. A second incubation was then carried out, and the vesicle contents of samples taken at different times were analyzed. After a 30 min incubation without any addition, approximately 85% of the preloaded radioactivity was still retained in the vesicles, and was recovered as ribose-1-P. Essentially the same results were obtained when unlabeled ribose-1-P was...

...
bution of radioactivity in [U-14C]inosine has been found (by hy-

drolysis of the radioactive nucleoside with commercial purine nu-

To monitor the effect of these compounds on the removal of the
intravesicular ribose-1-P, a second incubation was conducted, and the
vesicle content of samples taken at different times was analyzed.

Effectors are as follows: O, buffer only; ●, plus ribose-1-P; △,
plus hypoxanthine; ○, plus adenosine; ■, plus uracil.

added to the incubation medium at the beginning of the
reaction (Fig. 3). However, 1 mM hypoxanthine, adenosine, or
uracil in the incubation medium caused significant reduction of
intravesicular radioactive ribose-1-P, with hypoxanthine being
the most effective. Fig. 3 further shows that over a
concentration range in which the exogenous ribose-1-P added is
in great excess (100x or more) of the labeled ribose-1-P in the
medium, no inhibition of initial accumulation of intravesicular
ribose-1-P was maximized. The distri-

The dramatic inhibitory and efflux effect of hypoxanthine,
however, the other product of the phosphorolytic cleavage of
inosine, suggested the nucleoside phosphorylase might be
involved in the transport step. The similar effect of uracil,
which is not likely to interact with the same phosphorylase (cf.
data of accompanying paper, Ref. 17), shows that ribose-1-P was
probably free in the intravesicular space and not simply
bound to the inosine phosphorylase enzyme. Thus, if phos-
phorolysis did not precede transport, did it follow the transport
substrate for the accumulation of ribose-l-P, in-

Fig. 3. Effects of ribose-1-P, ribose-5-P, and hypoxanthine on
inosine transport. Concentration of radioactive inosine in the
incubation mixture was 12 μM, incubation time was 2 min. concentration
range of the effectors is as indicated. Other conditions were as specified
under “Materials and Methods.” V/V, is the ratio of transport
activities in pmol/mg of protein in the presence and absence of the
effectors. ●, ribose-1-P as effector; ○, ribose-5-P as effector; △,
hypoxanthine as effector.

centrifugation at 20,000 × g for 20 min was resuspended in the
standard buffer containing 5 μM [U-14C]inosine and 20 mM
caffeine. Caffeine was added because there was a great
difference observed in its effect on transport compared to its
effect on phosphorolysis (Table III). Because at 20 mM it
completely inhibited transport, while not having the same
effect on phosphorolysis, we thought it might aid in the
determination of whether intravesicular inosine was a sub-
strate for the phosphorolysis. Under these conditions, we could
follow the fate of intravesicular inosine in the presence of
extravesicular inosine without the latter being able to undergo
a transport process. A control sample without the supplement
of caffeine was also prepared. Even though the whole process of
homogenization and resuspension was conducted at low tem-
perature (4°) and for a relatively short time (about 30 min),
the action of the phosphorylase could not be completely abrogated,
since approximately 8% of the phosphorylase activity is
expressed at this temperature. Thus, after homogenization and
resuspension, the vesicles contain both inosine and ribose-1-P
rather than inosine alone. Nevertheless, we were able to
achieve a sufficiently high level of intact inosine to monitor its
fate. The ribose-1-P to inosine ratio achieved is 2, while that
found in the regular transport assay is usually greater than 10.

The control sample, where no caffeine was added, gave a
ribose-1-P to inosine ratio of 3.5 (points at zero time in Fig.
4B). The next step of this experiment was to subject these
inosine-loaded vesicles to a second incubation in order to
determine whether the intravesicular inosine is degraded to
ribose-1-P and hypoxanthine. If intravesicular inosine were
degraded then it would be possible that normally the transport
step precedes the cleavage step, and hypoxanthine might not
appear in the vesicles because of some exit process. The results
shown in Fig. 4A indicate that the phosphorylase is actually
not available to preloaded intravesicular inosine. In contrast,
the ribose-1-P to inosine ratio of the control sample, where no
caffeine was present and the external inosine could serve as the
transport substrate for the accumulation of ribose-1-P, in-
creased with time during the second incubation. This increase
represented greater accumulation of ribose-1-P rather than loss
of inosine. In both cases, the hypoxanthine level inside the
vesicles was insignificant. Hypoxanthine accumulated in the
extravesicular medium.

Thus, inosine is not a substrate for phosphorolysis when it is
inside the vesicles, while inosine still outside the vesicles, if
Table III

Effect of caffeine and 6-N-methylaminopurine riboside (6-methyladenosine) on transport and inosine phosphorolysis

The standard transport assay conditions were used for these experiments except for the presence of the inhibitors. The difference between these two assays is, therefore, that while the transport assay can only detect the accumulation of ribose-1-P intravesicularly, the phosphorylase assay would represent the total amount of ribose-1-P found in the vesicles and the surrounding medium. Numbers in parentheses indicate percentage of inhibition.

| Substrate used was inosine, i.e. ribose-1-P accumulated inside the vesicles, while hypoxanthine was the major product in the surrounding medium. Apparently, the adenosine deaminase must be involved in the membrane handling and possibly the transport process. This could mean either that the adenosine deaminase exists on the membrane surface in such excess that we were simply measuring inosine as transport substrate, or that deamination is actually a prerequisite for adenosine uptake.

One approach to the question of whether intravesicular accumulation of ribose-1-P from adenosine is necessarily preceded by deamination of the nucleoside would be the use of cells which are devoid of membrane-associated adenosine deaminase activity. In our collection of sublines of the L-cells, we found a facsimile in the A6 (19) mutant subline. The membrane preparation from this subline is found to exhibit only 4% of the adenosine deaminase activity of the L cells—vesicles. In addition, we found that the membrane-associated activities of adenosine deaminase of a revertant of A6 (A6) and a normal subline (L10A) from the Flow Laboratories fall in between. Table IV compares the activities of adenosine deaminase in the membrane vesicles of these sublines and the transport activities of these membrane preparations for the accumulation of ribose-1-P from adenosine and inosine. The results suggest that the activities for deamination and transport are correlated. The results in Table V show that the ratio of ribose-1-P inside the vesicles to that in the surrounding medium appears to be inversely related to the adenosine deaminase activity of the membrane preparation in which the transport process is carried out. It appears that each molecule of adenosine deaminated by the membrane vesicles with the least deaminase activity has a greater chance of undergoing vectorial phosphorolysis leading to the intravesicular accumulation of ribose-1-P, whereas in membrane vesicles with higher adenosine deaminase activity, the inosine formed appears more likely to appear in the surrounding medium and result from nonvectorial phosphorolysis, hypoxanthine and ribose-1-P appearing in the extravesicular medium. When inosine was used as the transport substrate, however, the ratio of ribose-1-P inside the vesicles to that outside is relatively constant among the four sublines. Thus, the data cannot be explained by the occurrence of more sealed vesicles in cell lines having less deaminase.

Phosphoribosyltransferase and Transport As shown in Fig. 1 and Table I, the monophosphate esters are part of the products of nucleoside transport found in the vesicles. It has been shown that association of nucleoside kinase activity with the membrane vesicles is insignificant (Table II), and no increase in enzyme activity is observed even in the presence of Triton X-100. Furthermore, in the presence of ATP or GTP, no increase in uptake of nucleoside radioactivity has ever been observed. Thus, phosphorylation of the nucleoside by the kinase during the transport process in the vesicles is unlikely. The alternative pathway for the formation of these monophosphate esters is through mediation of phosphoribosyltransferases. However, using the bases hypoxanthine or adenosine as transport substrates for the L-cell vesicles, we have never been able to demonstrate any significant uptake. This is in contrast to other cell lines studied in the laboratory (20, 21). Occasionally, when nucleosides specifically labeled in the base moiety were used in the transport assay, significant uptake did result. There is poor reproducibility of these results, however, for membrane vesicles from this cell line. We attribute these
Enzymes Involved in Vesicle Transport

discrepancies to the disruption of transport sites during membrane isolation rather than to differences in cell lines in situ, since the L<sub>1929</sub> cells do take up these purine bases efficiently (Fig. 5). It is highly probable that the sites for transport of the purine bases in most membrane vesicles prepared by the present methods may be sufficiently disrupted that vectorial phosphoribosylation is no longer carried out, yet the enzyme associated with the membrane is still capable of catalysis (nonvectorial action, cf. Ref. 16). Since uptake of the base moiety when it occurs at all in these vesicles is the base of nucleoside and never the free base, it is possible that only internal ribose-1-P can serve as precursor energy source. It is also possible that phosphorylase-bound base that originates from nucleoside is the preferred substrate for the phosphoribosyltransferases, as was observed for prokaryote membranes (14).

Finally, neither intact cells nor vesicles took up free pyrimidine bases. Thus, the small amount of uridine phosphorylase-mediated uptake of ribose-1-P from uridine (Fig. 1) in these cell membranes would represent that low level activity often observed in certain cell lines.

Alternative Routes of Adenosine Uptake in Intact Cells—Although the adenosine deaminase correlation made above indicates the possibility that deamination and phosphorolysis followed by phosphoribosylation might be the major route of adenosine utilization, another mechanism is quite active in intact cells. Fig. 6 shows that A<sub>5</sub> cells lacking both adenine and hypoxanthine-guanine phosphoribosyltransferases and adenosine and hypoxanthine uptake ability are able to take up [8-<sup>14</sup>C]adenosine. They cannot, however, take up [8-<sup>14</sup>C]inosine (Fig. 7), and only take up the ribose moiety of [U-<sup>14</sup>C]inosine, leaving behind labeled hypoxanthine in the medium at the same rate at which the ribose moiety accumulates in the cells. Also, ribose-1-P added to the medium did not inhibit uptake. When the rate at which radioactivity from [U-<sup>14</sup>C]inosine is incorporated into A<sub>5</sub> cells (the revertant cell line capable of utilization of both the sugar and the base moieties of inosine) is compared to the rate at which the ribose moiety is used in A<sub>5</sub> cells, the data suggest that the phosphorylase-mediated uptake is the predominant or only route of inosine utilization. Thus, there appears to be an alternate mechanism for adenosine utilization in these cells, but only a single major route for inosine utilization, which is as we have described above for the vesicles.

**DISCUSSION**

The data presented indicate that ribose-1-P is the predominant intravesicular product to accumulate in plasma membrane vesicles from L-cells when they are exposed to a variety of nucleosides (Fig. 1). Ribose-1-P itself is not the uptake substrate; thus, uptake does not follow phosphorolysis. This is shown in Figs. 2 and 3, and that intravesicular inosine is not a substrate for phosphorolysis is shown in Fig. 4. This leads to the conclusion that uptake and phosphorolysis occur at the same time, and thus suggests a group translocation-type transport mechanism where the phosphorilase and the transport-carrier are one in the same membrane components.

Although other data from this laboratory (4, 5) showed that phosphorolysis did not precede uptake of the ribose moiety in 3T3 membranes, the data reported here in Fig. 4 represent more direct evidence that phosphorolysis does not occur subsequent to uptake. Our finding for these membranes that uridine is cleaved at the membrane is in contrast to our findings for 3T3 membranes, which take up uridine intact (4, 5, 22). The fact that free uracil is not taken up, that this uridine phosphorilase activity is low, and that the cells are rigorously tested and found to be mycoplasma-free, suggests that this enzyme is a plasma membrane constituent of certain normal cell lines. Purine bases, although not taken up by the L cell vesicle preparations are taken up by intact cells (Fig. 5),

**TABLE IV**

<table>
<thead>
<tr>
<th>Reaction measured</th>
<th>L-cell sub-line</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L&lt;sub&gt;1929&lt;/sub&gt;</td>
<td>L&lt;sub&gt;193&lt;/sub&gt;</td>
<td>A&lt;sub&gt;5&lt;/sub&gt;</td>
<td>A&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Adenosine deaminase</td>
<td>100</td>
<td>70</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Transport: ribose-1-P accumulation from adenosine</td>
<td>100</td>
<td>41</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>Transport: ribose-1-P accumulation from inosine</td>
<td>100</td>
<td>79</td>
<td>83</td>
<td>76</td>
</tr>
</tbody>
</table>

**TABLE V**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>L&lt;sub&gt;1929&lt;/sub&gt;</th>
<th>L&lt;sub&gt;193&lt;/sub&gt;</th>
<th>A&lt;sub&gt;5&lt;/sub&gt;</th>
<th>A&lt;sub&gt;4&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V&lt;sup&gt;*&lt;/sup&gt;</td>
<td>M&lt;sup&gt;*&lt;/sup&gt;</td>
<td>V/M</td>
<td>V&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adenosine</td>
<td>53.0</td>
<td>5.30</td>
<td>10.3</td>
<td>17.6</td>
</tr>
<tr>
<td>Inosine</td>
<td>64.1</td>
<td>2.8</td>
<td>22.6</td>
<td>49.9</td>
</tr>
</tbody>
</table>

<sup>*</sup>V, vesicles; M, medium.
Finally, other data in the paper again point to the likelihood that enzymes which are easily solubilized on cell disruption may have membrane function in situ, and that such enzymes may have altered activity and response to inhibitors upon solubilization (cf. Ref. 5).

REFERENCES

Membrane-associated enzymes involved in nucleoside processing by plasma membrane vesicles isolated from L929 cells grown in defined medium.

C C Li and J Hochstadt


Access the most updated version of this article at [http://www.jbc.org/content/251/4/1181](http://www.jbc.org/content/251/4/1181)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/251/4/1181.full.html#ref-list-1](http://www.jbc.org/content/251/4/1181.full.html#ref-list-1)