Myocardial Nucleotide Synthesis
from Purine Bases and Nucleosides

COMPARISON OF THE RATES OF FORMATION
OF PURINE NUCLEOTIDES FROM VARIOUS PRECURSORS
AND IDENTIFICATION OF THE ENZYMATIC ROUTES
FOR NUCLEOTIDE FORMATION IN THE ISOLATED RAT HEART

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ABSTRACT

14C-Labeled adenosine, inosine, hypoxanthine, and adenine were extracted by the isolated rat heart in amounts proportional to their concentration in the perfusion medium between 0.05 and 5 μM. With each of the precursor materials, nearly all of the radioactivity retained by the heart was identified as acid-soluble nucleotide. Nucleotide formation from the four isotopic precursors occurred at similar rates when the concentration of the precursors was below 1 μM. Above this concentration, the heart appeared to utilize adenosine for nucleotide synthesis at rates three to five times those for the other purines. Several experimental approaches were employed to determine the predominant enzymatic routes in the rat heart for the conversion of the nucleosides adenosine and inosine to nucleotides. The results indicated that adenosine was directly phosphorylated to 5'-adenosine monophosphate by a nucleoside kinase. Inosine appeared to proceed to the nucleotide, at least partially, through an initial conversion to hypoxanthine.

KEY WORDS  cardiac nucleotides  adenosine  inosine  hypoxanthine  adenine
adenosine kinase  nucleotide synthesis

Extensive analysis of the degradation of adenine nucleotides within the myocardium has revealed that the major pathway of catabolism is adenosine triphosphate (ATP)→adenosine diphosphate (ADP)→adenosine monophosphate (AMP)→adenosine→inosine→hypoxanthine (1-3). The nonnucleotide catabolic products of this scheme readily leave the heart cells and are found in the coronary vascular space (4-6). It has been demonstrated that adenine nucleotide degradation occurs in the myocardium whenever the energy metabolism of the heart is restricted (e.g., hypoxia, substrate deprivation) and that a decrease in the tissue content of purine nucleotides in the heart is associated with this degradation (1, 2, 7, 8). Recently Rubio and Berne (6) have presented evidence which suggests that the production of nucleosides from the degradation of ATP may occur continually within the normal myocardium, resulting in a continual exit of some purines from the heart cells. These investigators have implicated the formation of a specific nucleoside, adenosine, in the regulation of coronary blood flow in both normal and hypoxic myocardium. My interests have led to an examination of the means by which cardiac muscle maintains its purine pool in view of the apparent leakage of nucleosides and bases from myocardial cells secondary to the catabolism of their nucleotides. The de novo synthesis rates of purine nucleotides in the heart have been shown to be low relative to the total cardiac nucleotide pool (9, 10). The alternative mechanism for maintenance of purine nucleotides is salvage of purine nucleosides and bases. This report deals with two characteristics of the salvage pathways. First, I examined the rate of nucleotide formation from radiolabeled adenosine, inosine, hypoxanthine, and adenine over a wide concentration range, and then I attempted to determine the enzymatic routes by which these precursors are utilized by the rat heart for nucleotide synthesis.

There has been no systematic study comparing the rates of cardiac nucleotide synthesis from a number of purine precursors. Liu and Feinberg (11) compared the rates of nucleotide formation
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from radioisotopic adenosine and inosine in the perfused rabbit heart and showed that adenosine was utilized at a faster rate. However, their study was carried out at only one perfusate concentration of the precursors and, thus, provided only limited information on which to base a comparison. Tsuboi and Buckley (12) demonstrated that adenine and hypoxanthine were incorporated into heart nucleotides, but they did not compare the rate or the extent of these processes.

Considerably more attention has been given recently to the study of the enzymatic routes of nucleotide formation in cardiac muscle. The identification of the preferred enzymatic routes is essential before a detailed biochemical study of the regulation of these processes is possible. In studies of nucleotide formation in the pig heart by Coldthwait (9) and the rabbit heart by Liu and Feinberg (11), it was concluded that a direct phosphorylation of adenosine was the most likely reaction occurring in cardiac muscle. Wiedmeier et al. (13) recently tested this premise by a more direct approach, and the conclusions they reached were consistent with the direct phosphorylation hypothesis. However, Maguire et al. (14) have reported evidence which they have interpreted to indicate that in the rat heart adenosine is preferentially converted to inosine, then to hypoxanthine, and finally to the nucleotide, 5'-hypoxanthine monophosphate (IMP) and that little adenosine proceeds to nucleotide by direct phosphorylation.

The studies presented in the present paper attempted to apply a direct test to the question of the adenosine pathway in the rat heart. A recent report by Wiedmeier et al. (15) indicated that inosine proceeded through a hypoxanthine intermediate prior to its incorporation into nucleotides of the guinea pig heart. The present study also examined the pathways for the utilization of inosine by the rat heart.

Methods

Sprague-Dawley rats (250–350 g) were heparinized (1000 units, ip) 20–30 minutes prior to anesthesia. The rats were anesthetized with ether, and their hearts were rapidly excised and washed in perfusion medium at 0°C. Hearts were then perfused by the Langendorff technique except that retrograde aortic flow was maintained constant at 10 ml/min by inserting a pump between the perfusion reservoir and the aortic cannula. This perfusion technique and the perfusion medium have been described in detail previously (16). The perfusion medium was maintained at 35°C. Hearts were frozen using Wollenberger clamps at the temperature of liquid N\textsubscript{2}. The perfusion medium, containing 14C-purine bases or nucleosides, was not recirculated and was collected on exit from the heart into vessels chilled in a Dry Ice-alcohol bath. All hearts were equilibrated for 30 minutes before commencing the infusion of the radioisotope. 3H-Sorbitol was also present in the perfusion medium to determine the extracellular space; this space was subtracted from the 14C-space to calculate 14C-precursor uptake. Uptake and nucleotide synthesis rates from the 14C-precursors were calculated based on the specific activity of the precursor in the perfusion medium and, thus, did not take into account any dilution of the specific activity by endogenous materials within the heart or its extrastitial.

Frozen hearts were homogenized in 0.5x perchloric acid containing 1 mM ethylenediaminetetraacetate (EDTA) at 0°C, and the perchloric acid extract was maintained at 0°C throughout the remainder of the procedure. The perchloric acid-insoluble material was sedimented by centrifugation and, after removal of the supernatant fluid, the sediment was washed three times in cold perchloric acid and dissolved in 1x NaOH. A sample of the digested acid-insoluble pellet was counted in a Packard liquid scintillation counter. The perchloric acid-soluble fraction of the heart was neutralized with KHCO\textsubscript{3} and assayed for ATP, ADP, and AMP by fluorometric procedures as described by Lowry et al. (17). A sample of this fraction was also counted to ascertain the total acid-soluble radioactivity, and another sample was applied to a small Dowex-1-C\textsubscript{18} column to batch-separate nucleotides from bases and nucleosides to estimate the percent of the labeled material in the form of heart acid-soluble nucleotide. In selected experiments, samples of the acid-soluble extracts were chromatographed on polyethyleneimine-cellulose thin-layer chromatography plates in two dimensions to separate the purine compounds of interest. The first development was in 0.1x NaCl, and the second dimension was developed in 1.0x NaCl. In each thin-layer chromatography experiment, standards containing 10 nmoles each of the purine nucleotides, nucleosides, and bases of interest were added to the extract to be chromatographed. Spots detected under ultraviolet light were cut out and eluted in 0.1x HCl and counted.

Purine nucleosides and bases present in the perfusate were concentrated by addition of acid-washed Norit (3–10 mg/40 ml perfusate). This step was carried out at 0°C, since the perfusate contained some adenosine deaminase activity. The Norit was collected by centrifugation, and a 10% aqueous pyridine solution was added to elute the adsorbed purine compounds. By careful titration of the amount of Norit used in this procedure, so as to not employ a large excess, 75–90% recoveries of added standards of adenosine, inosine, or hypoxanthine were attained. The eluate from Norit was chromatographed in one dimension on polyethyleneimine-cellulose (0.1x NaCl), and radioactivity was measured on a Actigraph III radiochromatograph scanner (Nuclear Chicago).

3H and 14C in neutralized, perchloric acid extracts were counted simultaneously in a liquid scintillation counter with efficiencies of about 22% and 52%.
respectively, and a spillover of $^{14}$C into the $^{3}$H window of about 12%. Efficiencies and spillover were calculated for each sample by the use of an external standard. The sorbitol space of the frozen heart samples averaged 0.42 ml/g with a range of 0.35 to 0.51 ml/g.

**MATERIALS**

The majority of experiments were done using nucleosides and bases labeled with $^{14}$C at the C-8 position on the purine ring, and these radioisotopes were supplied by Amersham/Searle. $^{3}$H-Sorbitol was obtained from New England Nuclear Corporation. Double-label experiments employed uniformly labeled $^{14}$C-adenosine also from Amersham/Searle and 2,8-$^{3}$H-adenosine obtained from New England Nuclear Corporation. Unlabeled nucleotides, nucleosides, and bases were obtained from P-L Biochemicals.

**Results**

Myocardial Uptake of $^{14}$C-Labeled Precursors of Purine Nucleotides.—The myocardial extraction of the radioisotopes of adenosine, adenine, inosine, and hypoxanthine was linear for up to 30 minutes. Figure 1 illustrates the time course of the extraction of adenosine and inosine at various perfusate concentrations. Most of the studies of uptake and nucleotide formation were done after a 30-minute perfusion of the isolated heart with the radioisotopic precursor.

The distribution of radioactivity between acid-soluble and acid-insoluble fractions of the heart extract is summarized in Table 1. After a 30-minute uptake period, 90% or more of the radioactivity was localized in the acid-soluble fraction, indicating little incorporation of the purine into nucleic acids in this time period. The amount of labeled purine material found in the acid-soluble fraction increased as the perfusate concentration of the precursor was raised, and almost all of this radioactive material within the heart was characterized as nucleotide by its retention on an anion-exchange column (Table 1). The amount of $^{14}$C-nucleotide formed from the four precursors as a function of their perfusate concentration is illustrated in Figure 2. Since I did not examine the uptake of each of the precursors at identical concentrations, a statistical comparison of the rates of nucleotide formation was not done. However, clearly below a concentration of 1 $\mu$M there was not a large difference between the rate of $^{14}$C-nucleotide formation from each of the purine materials. Above a perfusate concentration of 1 $\mu$M, adenosine was incorporated into heart nucleotides at a rate three to five times faster than that for the other precursor materials.

When uptake studies with $^{14}$C-adenosine were carried out at concentrations up to 20 $\mu$M, the rate of nucleotide formation appeared to exhibit saturation kinetics (Fig. 3). This pattern could not be confirmed, however, since higher perfusate concentrations of adenosine were toxic to the heart. In these experiments, I also followed the appearance of deaminated metabolites of adenosine in the perfusate. The total amount of deaminated metabolites (predominantly inosine) formed during a 30-minute perfusion was proportional to the concentration of the precursor in the perfusate (Fig. 4). The amount of unchanged adenosine in the perfusate leaving the heart expressed as a percent of the total radioactivity of the perfusate was considerably increased between 1 and 10 $\mu$M. Thus, the actual concentration of adenosine presented to the heart may have been reduced considerably by deamination, and this effect would be more pronounced at the lower adenosine concentrations.
Characterization of Radioactive Substances Found in Hearts Perfused with \(^{14}C\)-Labeled Adenosine and Adenine.—Neutralized extracts of hearts perfused with adenosine or adenine, 0.05 \(\mu M\) were applied to a thin layer of polyethyleneimine-cellulose (Brinkman) and developed in two dimensions to resolve many of the naturally occurring purine bases, nucleosides, and nucleotides. In extracts of hearts perfused with these low concentrations of \(^{14}C\)-labeled precursor, more than 90% of the radioactivity of the extracts was accounted for in seven spots identified in Tables 2 and 3. The sum of the radioactivity from these seven areas on the chromatogram was taken as 100% when calculating the percent of total radioactivity represented by these compounds (Tables 2 and 3). When either \(^{14}C\)-adenosine or \(^{14}C\)-adenine was used, the major fraction of the radioactivity of the heart extract co-chromatographed with ATP (48.8% for \(^{14}C\)-adenosine and 63.8% for \(^{14}C\)-adenine). This incorporation amounted to only a small ATP synthesis, considerably less than 0.1% of the total ATP content of heart tissue. The amount of \(^{14}C\)-ADP and \(^{14}C\)-AMP formed was less than that of \(^{14}C\)-ATP, but the smaller pool sizes of these adenine nucleotides were responsible for the higher specific activities observed for these compounds. These studies con-
Rates of nucleotide formation at high concentrations of adenosine. Each point represents three hearts analyzed in duplicate.

confirmed our earlier observations that the greatest fraction of the labeled material retained by the heart was in the form of nucleotide.

Enzymatic Pathways for Heart Nucleotide Synthesis.—The purine bases hypoxanthine and adenine were presumably incorporated into nucleotides via phosphoribosylation utilizing phosphoribosyl pyrophosphate (PRPP) in the presence of their respective phosphoribosyltransferases (18, 19) (steps 3 and 6, Fig. 5). However, more than one route of nucleotide synthesis was possible when the precursor material was the riboside of these two bases. First, both nucleosides could be phosphorolyzed to their corresponding purine bases (steps 2 and 5) and then proceed, through PRPP transferases, to the nucleotide level. Alternatively, both nucleosides could be directly phosphorylated by ATP in the presence of a nucleoside kinase (steps 1 and 9). Finally, adenosine could be deaminated to inosine and then be incorporated into nucleotide by either of the two possible routes for the 6-hydroxy-nucleoside as detailed above. It is important to note that steps 2 and 9 are only hypothetical, since neither a nucleoside kinase with high affinity for inosine nor a nucleoside phosphorylase with high affinity for adenosine have been found in mammalian tissues.

One approach used to delineate the predominant pathway of nucleotide synthesis from purine nucleosides was to determine what effect, if any, the addition of an unlabeled potential intermediate in the pathway produced on ^14C-nucleotide synthesis. The rationale for this test was that if, for example, ^14C-inosine proceeded to the nucleotide level via conversion to hypoxanthine (steps 5 and 6,
Fig. 5), the addition of unlabeled hypoxanthine to the perfusion medium would dilute the specific activity of the $^{14}$C-hypoxanthine pool and, thus, lower the rate of $^{14}$C-nucleotide synthesis. The results of such additions on the incorporation of $^{14}$C-adenosine and $^{14}$C-inosine into heart nucleotides are summarized in Table 4. The 30-minute labeling of heart nucleotides by $^{14}$C-adenosine was not significantly altered by addition of cold inosine, hypoxanthine, and adenine. $^{14}$C-Inosine incorporation into heart nucleotides was significantly lowered by the addition of unlabeled hypoxanthine to the perfusion medium.

A more direct test of the route of nucleotide synthesis from adenosine was also employed. Adenosine was added to the perfusion medium labeled with $^3$H in the 2 and 8 positions of the purine moiety and uniformly labeled with $^{14}$C. The final perfusate concentration was adjusted with cold adenosine to yield 0.005, 0.05, or 0.5 $\mu$m. $^3$H-Sorbitol was omitted from the experiment. The $^3$H-$^{14}$C ratio of perfusate adenosine and of heart nucleotides was determined. The rationale of these experiments was that if the major route of nucleotide synthesis from adenosine was through a pathway other than direct phosphorylation (step 1, Fig. 5) a phosphorolysis step would cause the loss of some of the $^{14}$C in the final nucleotide pool with respect to the amount of $^3$H incorporated, i.e., the final $^3$H-$^{14}$C ratio of the nucleotide pool would be greater than that of perfusate adenosine. To test the uniformity of the $^{14}$C label, a $^3$H-$^{14}$C-adenosine sample was acid-hydrolyzed and chromatographed on polyethyleneimine-cellulose; the adenine spot was eluted and counted for $^3$H and $^{14}$C (Table 5). The nucleotide fraction of the perchloric acid extracts of hearts perfused with $^3$H-$^{14}$C-adenosine exhibited $^3$H-$^{14}$C ratios of 15-16. The slight decrease

### Table 2

Fate of $^{14}$C-Adenosine Incorporated into the Perfused Rat Heart

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Tissue content (pmoles/g)</th>
<th>$^{14}$C-incorporation (pmoles/g)</th>
<th>% Endogenous pool</th>
<th>% Total radioactivity incorporated</th>
<th>Specific activity (pmole/µmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>4.84</td>
<td>0.83</td>
<td>0.017</td>
<td>48.8</td>
<td>0.009</td>
</tr>
<tr>
<td>ADP</td>
<td>0.34</td>
<td>0.42</td>
<td>0.12</td>
<td>24.5</td>
<td>0.056</td>
</tr>
<tr>
<td>AMP</td>
<td>0.038</td>
<td>0.19</td>
<td>0.58</td>
<td>11.5</td>
<td>0.298</td>
</tr>
<tr>
<td>Adenosine</td>
<td>X</td>
<td>0.072</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Inosine</td>
<td>X</td>
<td>0.074</td>
<td>X</td>
<td>4.3</td>
<td>X</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>X</td>
<td>0.032</td>
<td>X</td>
<td>1.9</td>
<td>X</td>
</tr>
<tr>
<td>Adenine</td>
<td>X</td>
<td>0.061</td>
<td>X</td>
<td>3.6</td>
<td>X</td>
</tr>
</tbody>
</table>

The rat heart was perfused for 40 minutes with $5 \times 10^{-8}$M adenosine, specific activity 52 pmole/µmole. Total uptake = 1.71 nmoles/g. X indicates not measured. Adenosine uptake was corrected for extracellular adenosine. Percent of endogenous pool was calculated as (tissue content of $^{14}$C-nucleotide/total tissue content of nucleotide) X 100. These data are derived from triplicate analyses of one perfused heart and are typical of results observed in two additional such experiments.

### Table 3

Fate of $^{14}$C-Adenine Incorporated into the Perfused Rat Heart

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Tissue content (pmoles/g)</th>
<th>$^{14}$C-incorporation (pmoles/g)</th>
<th>% Endogenous pool</th>
<th>% Total radioactivity incorporated</th>
<th>Specific activity (pmole/µmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>5.14</td>
<td>1.6</td>
<td>0.03</td>
<td>63.8</td>
<td>0.086</td>
</tr>
<tr>
<td>ADP</td>
<td>0.48</td>
<td>0.74</td>
<td>0.15</td>
<td>29.0</td>
<td>0.428</td>
</tr>
<tr>
<td>AMP</td>
<td>0.044</td>
<td>0.14</td>
<td>0.32</td>
<td>5.8</td>
<td>0.88</td>
</tr>
<tr>
<td>Adenosine</td>
<td>X</td>
<td>0.004</td>
<td>X</td>
<td>0.15</td>
<td>X</td>
</tr>
<tr>
<td>Inosine</td>
<td>X</td>
<td>0.015</td>
<td>X</td>
<td>0.6</td>
<td>X</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>X</td>
<td>0.001</td>
<td>X</td>
<td>0.05</td>
<td>X</td>
</tr>
<tr>
<td>Adenine</td>
<td>X</td>
<td>0.013</td>
<td>X</td>
<td>0.5</td>
<td>X</td>
</tr>
</tbody>
</table>

The rat heart was perfused for 40 minutes with $5 \times 10^{-8}$M adenine, specific activity 278 pmole/µmole. Total uptake = 2.54 nmoles/g. X indicates not measured. These data were derived and calculated as stated in Table 2.
Metabolic pathways of purines. Numbered reactions are catalyzed by the following enzymes: (1) nucleoside kinase, (2) purine nucleoside phosphorylase, (3) adenine phosphoribosyltransferase, (4) adenosine deaminase, (5) purine nucleoside phosphorylase, (6) hypoxanthine phosphoribosyltransferase, (7) adenylosuccinate synthetase, (8) adenylosuccinase, and (9) nucleoside kinase. Inclusion in the diagram does not necessarily indicate that these reactions have been shown to occur in heart muscle. PRPP = 5-phosphoribosyl-1-pyrophosphate, P_i = inorganic phosphate, ATP = adenosine-5'-triphosphate, and GTP = guanosine-5'-triphosphate.

in the ^3_H-^14_C ratio in heart nucleotides was difficult to rationalize biochemically, and thus I chose to examine whether the decrease could have been caused by exchange of some of the ^3_H-purine with H_2O as has been reported to occur under certain conditions by Tomasz et al. (20). ^3_H-Adenosine, 0.05 μM, was warmed to 35°C (the thermal conditions of the perfusion experiments), the solution was distilled in vacuo at this temperature, and the distilled H_2O was collected and counted for ^3_H. I found that, under these conditions, approximately 15% of the ^3_H from the purine had exchanged with the water of the medium. Thus the true value for the nucleoside isotopic ratio was calculated to be 15.5 (Table 5). This value is close to that actually observed for the ^3_H-^14_C ratio of the rat heart nucleotide pool.

**Discussion**

The isolated rat heart was shown to extract radioisotopically labeled adenosine, adenine, inosine, and hypoxanthine from the perfusion medium and to incorporate the label into the acid-soluble nucleotide pool of the heart. The observation that nearly all of the radioactivity retained by the heart was in the form of acid-soluble nucleotide is similar to results obtained in previous studies which had restricted their attention to either the cardiac metabolism of adenosine (11, 21-24) or inosine (11, 15). One obvious discrepancy between the present results about the pattern of nucleoside utilization and those found in previous studies is the subject of the specific activities of the labeled cardiac adenine nucleotides after perfusion with ^14_C-adenosine. Liu and Feinberg (11) found, after a 30-minute perfusion with labeled adenosine, that the specific activity ratio for ATP:ADP:AMP was 3.3:2.9:1.0, but the present results gave quite the reverse ratio of 0.05:0.2:1.0. An explanation for this divergence of experimental results is not readily apparent. However, it should be noted that the earlier study reported a considerably higher cardiac content of ADP and AMP than was found in my study. This observation may indicate that there was some

**TABLE 4**

Effects of the Addition of Possible Intermediates on the Incorporation of Adenosine and Inosine into Heart Nucleotides

<table>
<thead>
<tr>
<th>^14_C-labeled precursor</th>
<th>Concentration (μM)</th>
<th>Addition</th>
<th>Concentration (μM)</th>
<th>Nucleotide synthesis (moles/g 30 min^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>None</td>
<td>0.05</td>
<td>1.34 ± 0.20</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Inosine</td>
<td>0.05</td>
<td>1.44 ± 0.14</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Inosine</td>
<td>0.5</td>
<td>1.27 ± 0.09</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Inosine</td>
<td>5.0</td>
<td>1.48 ± 0.15</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Adenine</td>
<td>0.05</td>
<td>1.28 ± 0.11</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Adenine</td>
<td>0.5</td>
<td>1.17 ± 0.10</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Hypoxanthine</td>
<td>0.05</td>
<td>1.22 ± 0.10</td>
</tr>
<tr>
<td>Adenosine</td>
<td>0.048</td>
<td>Hypoxanthine</td>
<td>0.5</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.047</td>
<td>None</td>
<td>0.05</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.047</td>
<td>Inosine</td>
<td>0.068</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>Inosine</td>
<td>0.047</td>
<td>Hypoxanthine</td>
<td>0.68</td>
<td>0.31 ± 0.03*</td>
</tr>
</tbody>
</table>

Synthesis values represent means ± SE for 4-6 hearts.
*Indicates differs from control at P < 0.01.
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TABLE 5
Isotope Ratio of 2,8-^H,14C(U)-Adenosine Incorporated into Heart Nucleotides

<table>
<thead>
<tr>
<th></th>
<th>2H:14C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original adenosine</td>
<td>17.9</td>
</tr>
<tr>
<td>Adenine from acid-hydrolysis of adenosine</td>
<td>30.4</td>
</tr>
<tr>
<td>Heart nucleotides</td>
<td></td>
</tr>
<tr>
<td>Expt. 1</td>
<td>16.2</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>15.1</td>
</tr>
<tr>
<td>Expt. 3</td>
<td>16.4</td>
</tr>
<tr>
<td>Theoretical value for nucleotides via kinase pathway</td>
<td>15.5</td>
</tr>
</tbody>
</table>

Experiment 1 done with adenosine concentration of 0.005 μM, 2 with 0.05 μM, and 3 with 0.5 μM. Theoretical value is based on correction for 2H exchange with water at 35°C (see text).

nucleotide catabolism prior to the assay procedure in the earlier study which would yield low apparent specific activities of the mono- and diphosphate nucleotides.

The comparison of the rates of label incorporation from the four precursor materials indicated that (1) below a concentration of precursor in the perfusion medium of 1 μM the amount of label incorporated into the nucleotide pool was very similar for all precursors and (2) above a medium concentration of 1 μM 14C-adenosine was incorporated into heart nucleotides more rapidly than were the other precursors. To accurately interpret these rates of isotope incorporation into nucleotides as representative of the rates or capacities for net cardiac nucleotide synthesis via these salvage pathways, it must be considered that the specific activity of the precursor materials within the heart may have been significantly affected by the pool size of endogenous cold precursor. Endogenous purine would dilute the specific activity of the isotopic precursor, and, thus, the estimation of nucleotide synthesis rates from isotope incorporation data would be necessarily low. This complication would be most significant for inosine and hypoxanthine, both of which are thought to be present in the heart and the cardiac interstitium in concentrations of up to 0.5 μM (5, 13). Thus, it can be assumed that the actual nucleotide synthesis rates from these two precursors given by the present data underestimate to some degree the true salvage capacity via these pathways. This problem of interpretation of synthesis rates is probably minimal for adenosine and adenine, because they probably are not present in the well-oxygenated, perfused heart in concentrations exceeding 0.05 μM (11, 13). A further complication in relating isotope incorporation rates to the actual capacity of the heart to utilize purines is that the metabolism of the precursor may influence the incorporation-concentration relationship. Such an effect is especially likely for adenosine, because the extent of the deamination of this nucleoside differs at different perfusate concentrations.

In spite of the complexities in interpreting isotope incorporation data in terms of nucleotide salvage rates, several conclusions can be drawn from the results with the four precursors. Synthesis of cardiac nucleotides by the salvage mechanisms takes place at rates of 0.1-0.5 nmoles/min g⁻¹ in the isolated rat heart with all four precursors at submicromolar concentrations. Although the initial nucleotide product formed by the salvage mechanism differs depending on the precursor, the process of phosphorylation represents the first step in preserving preformed purine for the myocardial metabolic process, and on this basis the differing salvage pathways can be equated. Thus, the present data suggest that the identity of the catabolic product of nucleotide degradation is probably not a major factor in determining the rate of purine reutilization by the myocardium. Only if the local interstitial concentration of nucleotide catabolic product should reach 1 μM or more, would the identity of the product be important in determining its reutilization, since it appears that adenosine is preferentially processed at these higher concentrations.

Allowing for the fact that some of the present nucleotide synthesis rates from radioactive precursors may be underestimates, it is possible to conclude from these findings that rates of salvage of preformed purines by the isolated rat heart may always exceed rates of de novo nucleotide synthesis. De novo synthesis rates, both in cell-free and intact myocardium, have been found to be about 0.02 nmoles/min g⁻¹ (9, 10). This value is well below the salvage rates from all four precursors at even the very lowest concentrations employed in the present experiments. Thus, these salvage mechanisms may be more important than de novo synthesis in the maintenance of cardiac nucleotide pools under normal conditions. De novo nucleotide synthesis is augmented after hypoxia and may become a more important contributor to nucleotide synthesis under such conditions (10). I do not, presently, have knowledge of any alterations in salvage rates under similar conditions of oxygen deprivation. It is interesting to note that although

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the salvage rates for the rat heart may exceed de novo synthesis rates both pathways of nucleotide formation are meager compared with the total adenine nucleotide pool of the heart. In fact, the salvage rates found in this study would only be capable of maintaining cardiac nucleotide levels in steady-state when the nucleotide breakdown does not exceed about 0.002% of the total nucleotide pool per minute. The observation of Pritchard (25) that the $^{14}$C-nucleotide pool of the heart in situ is very stable probably indicates that under normal conditions the salvage pathways are adequate to retard any net purine loss from the heart.

The present findings suggested that inosine was at least partially converted into hypoxanthine before this purine source reached the nucleotide level. The experiments that demonstrated the inhibition of nucleotide formation from $^{14}$C-inosine by the addition of cold hypoxanthine did not exclude the possibility that a portion of this nucleoside could have been incorporated into nucleotide by a direct phosphorylation reaction. However, the nucleoside kinase from rat heart has been reported not to utilize inosine as substrate (26), and this observation has recently been confirmed in my laboratory. Thus, it may be a reasonable assumption that all of the purine from inosine that is incorporated into heart nucleotides occurs as a result of the enzymatic activity of nucleoside phosphorylase and hypoxanthine PRPP transferase. This conclusion is in agreement with that reached by Wiedmeier et al. (15) in their studies with the guinea pig heart.

The experiments which examined the adenosine pathway in rat heart gave no indication that adenosine was metabolized to any intermediate before it was incorporated into nucleotides. The present tests indicated rather that adenosine was utilized for nucleotide synthesis exclusively through a nucleoside kinase reaction. This conclusion is similar to that derived from studies done with hearts from three other species, pig (9), rabbit (11), and guinea pig (13). Maguire et al. (14) concluded that the rat heart may utilize adenosine for nucleotide synthesis through a pathway other than that involving a direct phosphorylation reaction. Their conclusion was based on the low activity of adenosine kinase found in crude extracts of rat ventricular muscle. I believe that my results concerning the pathway in rat heart, which were derived from more direct tests and using intact cells, supercede those derived from the in vitro studies of Maguire et al. (14). Measurements of enzyme activities in vitro are often not readily extrapolated to intact cells, since they usually do not take into account factors which affect enzyme rates in situ. It should be noted that although the present data indicate that adenosine may be exclusively incorporated into heart nucleotides via direct phosphorylation this incorporation may be the case only under the present set of experimental conditions (i.e., nonrecirculated perfused heart). I have shown that the heart is capable of utilizing any of the metabolites of adenosine. In the heart in situ when adenosine production occurs as a result of nucleotide degradation it is likely that the deaminated metabolites of adenosine as well as the unmetabolized nucleoside are reutilized for nucleotide synthesis.

The present data suggest that, in the rat heart, the activity of both adenosine kinase and adenosine deaminase are important determinants of the amount of adenosine available within the myocardium and may determine the rate at which adenosine leaves the heart and the rate of its reuptake by heart cells.

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MYOCARDIAL NUCLEOTIDE SYNTHESIS


Myocardial Nucleotide Synthesis from Purine Bases and Nucleosides: Comparison of the Rates of Formation of Purine Nucleotides from Various Precursors and Identification of the Enzymatic Routes for Nucleotide Formation in the Isolated Rat Heart

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