The Utilization of Purines and Their Ribosyl Derivatives for the Formation of Adenosine Triphosphate and Guanosine Triphosphate in the Mature Rabbit Erythrocyte*

BERTRAM A. LOWY,† MARJORIE K. WILLIAMS, AND IRVING M. LONDON

From the Departments of Medicine and Biochemistry, Albert Einstein College of Medicine, Yeshiva University, and the Bronx Municipal Hospital Center, New York 61, New York

(Received for publication, November 23, 1960)

The finding that the purine portions of both adenosine triphosphate and guanosine triphosphate of the mature rabbit erythrocyte are not metabolically stable constituents of that cell, as is the heme of hemoglobin, suggested the possibility of a metabolic absence of any detectable utilization of sodium formate-Cl4 or of renewal of the nucleotides during the life of the cell (1, 2). The absence of any detectable utilization of sodium formate-C14 or of glycine-2-C14, in vitro, for purine nucleotide synthesis de novo suggested that the complete pathway of purine nucleotide formation from the small molecule precursors was not intact in this cell (3). The incorporation of sodium formate-C14 into the purine portions of both ATP and GTP, in the presence of 5-amino-1-ribosyl-4-imidazolecarboxamide, indicated that the mature rabbit erythrocyte, in vitro, has the capacity for carrying out the final steps of the pathway of purine nucleotide synthesis (3–5).

Reports from several laboratories have suggested that tissues which lack the capacity for purine synthesis de novo, in vitro, may utilize preformed purines extensively for the formation of acid-soluble nucleotides and nucleic acids (6, 7). Lajtha and Vane (7) have proposed that an organ, such as the liver, which can synthesize purine nucleotides de novo may supply preformed purines to tissues, such as bone marrow, which have a limited capacity for purine ring formation but which may utilize preformed purines for purine nucleotide synthesis by the nucleotide pyrophosphorylase reaction (8, 9).

This paper is concerned with the utilization of free purines and of the glycones of purine nucleosides for the formation of the nucleoside triphosphates of adenine and guanine in the mature rabbit erythrocyte.

EXPERIMENTAL PROCEDURE

Incubation in Vitro—Suspensions of rabbit erythrocytes were prepared and incubations were carried out as described previously (2). The radiocarbon-labeled purines and ribosyl derivatives were obtained commercially. Dr. Ralph Barclay kindly provided the 6-diazo-5-oxo-norleucine (diazooxonorleucine).

Administration in Vivo—Adenine-8-C14 in 0.9% sodium chloride solution (0.92 mg of adenine per ml) was administered subcutaneously (200 µc, 68 pmoles) as single injections to two white rabbits of the New Zealand strain, weighing about 4 kg each. At different time intervals, the animals were exsanguinated by cardiac puncture and erythrocytes were prepared as previously described (2).

Preparation of Purines—In the experiments in vitro and in vivo acid-soluble nucleotides were extracted from washed erythrocytes with trichloroacetic acid. ATP and GTP, obtained by ion exchange chromatography of the extract on a column of Dowex 1-Cl, were hydrolyzed, the free purine bases were prepared, and the specific radioactivities of the adenine and guanine were determined.

RESULTS AND DISCUSSION

The data obtained (Table I) after the incubation of rabbit erythrocytes with several of the naturally occurring purines indicated that these compounds can serve, to varying degrees, as precursors of either ATP or GTP, or of both. Adenine-8-C14 was metabolized extensively to ATP. Guanine-8-C14 and xanthine-8-C14 were utilized for the formation of GTP, but xanthine served much less effectively than guanine. Hypoxanthine-8-C14 was converted to both ATP and GTP, and the relative incorporation into the purines of the nucleoside triphosphates was inversely proportional to the approximate pool sizes of the two compounds, as determined by the quantities eluted from a Dowex 1 ion exchange column. It is of interest to note that the utilization of purines for nucleotide formation provides evidence for the formation of 5-phosphoribosyl pyrophosphate within the erythrocyte.

In contrast to the metabolism of adenine-8-C14, adenosine-8-C14 was found to be utilized for both ATP and GTP formation. The relative incorporation of adenosine-8-C14 into the two purine bases was quite similar to that found for hypoxanthine-8-C14, for inosine-8-C14, and for sodium formate-C14 plus 5-amino-1-ribosyl-4-imidazolecarboxamide. These findings suggest that the major route of utilization of adenosine was via a deamination product. Rapid deamination of adenosine would be consistent with the widespread presence of adenosine deaminase in rabbit tissues (10), and would account for the utilization of adenosine for both adenine and guanine nucleotides, presumably via hy-
TABLE I
Utilization of purines and purine derivatives for formation of ATP and GTP in rabbit erythrocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Incubated*</th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Adenine-8-C(^{14})</td>
<td>0.69</td>
<td>8.0 \times 10^4</td>
</tr>
<tr>
<td>Hypoxanthine-8-C(^{14})</td>
<td>0.87</td>
<td>1.2 \times 10^5</td>
</tr>
<tr>
<td>Xanthine-8-C(^{14})</td>
<td>0.90</td>
<td>8.4 \times 10^4</td>
</tr>
<tr>
<td>Guanine-8-C(^{14})</td>
<td>0.47</td>
<td>1.2 \times 10^4</td>
</tr>
<tr>
<td>Adenosine-8-C(^{14})</td>
<td>1.5</td>
<td>4.8 \times 10^4</td>
</tr>
<tr>
<td>Inosine-8-C(^{14})</td>
<td>1.5</td>
<td>4.0 \times 10^4</td>
</tr>
<tr>
<td>Guanosine-8-C(^{14})</td>
<td>1.5</td>
<td>3.6 \times 10^4</td>
</tr>
<tr>
<td>Sodium formate-C(^{14}) and ribosyl AICA(^{\dagger})</td>
<td>1.5</td>
<td>4.7 \times 10^4</td>
</tr>
</tbody>
</table>

* Washed rabbit erythrocytes in isotonic sodium phosphate buffer (pH 7.2) incubated with glucose (150 \mu moles) for 3 hours at 38\(^\circ\) ± 1\(^\circ\).

† RSA = c.p.m. per \mu mole purine isolated \times 100.

\dagger 5-Amino-1-ribosyl-4-imidazolcarboxamide.

---

TABLE III
Metabolic stability of purine rings of nucleoside triphosphates of rabbit erythrocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Incubated*</th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Specific activity</td>
</tr>
<tr>
<td>Adenine-2-C(^{14}) (5 \mu c)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Adenine-2-C(^{14}) (5 \mu c)</td>
<td>15</td>
<td>75</td>
</tr>
</tbody>
</table>

* 25 ml of washed erythrocytes incubated in 25 ml of isotonic sodium phosphate buffer containing glucose (150 \mu moles) for 3 hours at 37\(^\circ\).

---

FIG. 1. Summary of reactions leading to purine nucleotides in the mature rabbit erythrocyte, in vitro

\[
\begin{align*}
\text{adenosine} & \rightarrow \text{inosine} \\
\text{4-amino-5-imidazolecarboxamide} & \rightarrow \text{hypoxyanthine} \\
\text{5-amino-1-ribosyl-4-imidazolcarboxamide} & \rightarrow \text{inosinic acid} \\
\text{xanthine} & \rightarrow \text{guanylic acid} + \text{GDP} + \text{GTP} \\
\text{guanosine} & \rightarrow \text{adenosine} \\
\text{adenylosuccinic acid} & \rightarrow \text{adenylic acid} + \text{ADP} + \text{ATP} \\
\end{align*}
\]

TABLE II
Effect of 6-diazo-5-oxo-L-norleucine on utilization of xanthine and guanosine for formation of ATP and GTP in rabbit erythrocyte

<table>
<thead>
<tr>
<th>Compound</th>
<th>Incubated*</th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific activity</td>
<td>DON\dagger</td>
</tr>
<tr>
<td>Xanthine-8-C(^{14})</td>
<td>3.6 \times 10^4</td>
<td>0</td>
</tr>
<tr>
<td>Xanthine-8-C(^{14})</td>
<td>3.6 \times 10^4</td>
<td>15</td>
</tr>
<tr>
<td>Guanosine-8-C(^{14})</td>
<td>3.0 \times 10^4</td>
<td>0</td>
</tr>
<tr>
<td>Guanosine-8-C(^{14})</td>
<td>3.0 \times 10^4</td>
<td>15</td>
</tr>
</tbody>
</table>

* 25-ml aliquots of washed rabbit erythrocytes in 25 ml of isotonic sodium phosphate buffer (pH 7.2) incubated with radiocarbon-labeled compound (22.5 \mu moles) and glucose (150 \mu moles) for 3 hours at 37\(^\circ\).

\dagger 6-Diazo-5-oxo-L-norleucine.

---

TABLE IV
Utilization of adenine-8-C\(^{14}\) for ATP and GTP formation in rabbit erythrocyte, in vivo

<table>
<thead>
<tr>
<th>Compound</th>
<th>Days after administration</th>
<th>Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>1.01</td>
</tr>
<tr>
<td>Adenine-8-C(^{14})</td>
<td>13</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* Specific activity: 1.03 \times 10^6 c.p.m. per \mu mole; administered 68 \mu moles in a single injection.

---

The utilization of guanosine-8-C\(^{14}\) was found to be similar to that of guanine-8-C\(^{14}\). Each compound was utilized effectively for guanine nucleotide formation, with a considerable net formation of GTP within the cell. The small amount of labeling in the adenine nucleotides may have arisen by a reductive deamination mechanism (12, 13).

The findings reported here are summarized in Fig. 1. The mature rabbit erythrocyte is capable of forming adenine and xanthine formed by phosphorolytic cleavage of inosine (11). However, the direct phosphorylation of inosine cannot be excluded (2) nor can the possibility that some utilization of the purines may occur by the initial formation of nucleosides. The small extent of utilization of adenine-8-C\(^{14}\) for GTP formation suggests a limited conversion of AMP to IMP.

Effect of 6-diazo-5-oxo-L-norleucine on utilization of xanthine and guanosine for formation of ATP and GTP in rabbit erythrocyte.
The inhibition by diazoxononucleine of guanine nucleotide formation from inosinic acid has been demonstrated (4). Since glutamine is required for the conversion of xanthinic acid to guanylic acid, it has been postulated that this is the site of inhibition (5, 14). When rabbit erythrocytes were incubated with guanosine-8-C¹⁴ the addition of the inhibitor had no significant effect on the extent of utilization for GTP formation (Table II). Guanosine was studied instead of guanine because of its greater solubility. Extensive phosphorolytic cleavage of guanosine to guanine by the purine nucleoside phosphorylase of the erythrocyte is known to occur. When erythrocytes were incubated with xanthine-8-C¹⁴ and diazoxononucleine, essentially complete inhibition of utilization of the xanthine for GTP formation occurred as would be expected in view of the mechanism of action of the inhibitor. The inhibition observed indicates that both guanine and guanosine are utilized for GTP formation without prior conversion to xanthine or a xanthine derivative. Inhibition of labeling of the guanine of GTP has also been shown when erythrocytes were incubated with 4-aminoo-5-imidazolecarboxamide and sodium formate-C¹⁴ (5), and with adenine-8-C¹⁴. The inhibition of the small amount of labeling of GTP from adenine-8-C¹⁴ by diazoxononucleine indicates that, although small, the labeling of GTP is significant.

The lability of carbon 2 of the purine ring has been observed in purine nucleotides (15, 16), and pyridine nucleotides (17). In order to study this lability, adenine-2-C¹⁴ was incubated with rabbit erythrocytes in the presence of a large excess of unlabeled sodium formate (Table III). The failure to observe a dilution of the label in the isolated adenine of ATP in the presence of unlabeled sodium formate indicates a stability of carbon 2 of the purine ring during the several reactions leading to the formation of nucleoside triphosphates from adenine. Although a lability of carbon 2 of the purine ring of inosinic acid has been demonstrated in other systems (16), it may be excluded in the rabbit erythrocyte since labeling of the purine ring fails to occur after incubation with sodium formates-C¹⁴ and the other purine ring precursors, even in the presence of unlabeled inosine (3), a precursor of inosinic acid. The complete absence of label in these experiments indicates the inability of rabbit erythrocytes to utilize sodium formate by either the over-all purine biosynthetic sequence de novo or by the inosinic acid exchange mechanism demonstrated by Flaks, Warren, and Buchanan (15).

The finding that adenine-8-C¹⁴ was used primarily for adenine nucleotide formation in the rabbit erythrocyte, in vitro, with only a small amount of labeling in the guanine nucleotides, suggested a limited conversion of adenosine 5'-phosphate to inosine 5'-phosphate. To compare the utilization in vivo of adenine-8-C¹⁴ with the results in vitro, the labeled compound was administered to several rabbits and, after 5- and 13-day intervals, the relative specific activities of the purine portions of ATP and of GTP of the erythrocyte were determined. It was found (Table IV) that both purines were labeled, and to about the same extent, at each time period. This finding raises several interesting points. In view of a paucity of adenase in most tissues, it would appear that the major portion of the adenine is metabolized directly to adenosine 5'-phosphate. Although the AMP thus formed may be converted to ADP and ATP and utilized for polynucleotide synthesis within the cell in which it is produced, it is unlikely that it enters the erythrocyte as a nucleoside phosphate because of the apparent impermeability of the cell to phosphorylated compounds. Furthermore, the findings in vitro suggest a limited conversion of AMP to IMP, the obligatory precursor of GMP. It is reasonable to suggest, therefore, that the AMP is converted to other compounds, presumably free purines or nucleosides, which then enter the erythrocyte and are converted to ATP and GTP. In view of the divergent labeling of the purines after incubation with adenine-8-C¹⁴, the most likely explanation for the equal relative specific activity values for the two purines, in vivo, would result from uptake by the cell of a mixture of purines or nucleosides, or of both (e.g. adenine and guanine, hypoxanthine or inosine and adenosine). It is of interest to note that the administration of glycine-2-C¹⁴ to a group of rabbits also resulted in ATP and GTP with approximately equal labeling in both purine portions (2). In both experiments in vivo it was also observed that specific radioactivity of the purines of the nucleoside triphosphates decreased to one-half from the fifth day to the thirteenth day after administration of labeled precursor.

**SUMMARY**

The mature rabbit erythrocyte is capable of utilizing purines and their ribosyl derivatives for nucleoside triphosphate formation. Adenine is used extensively for adenosine triphosphate synthesis while xanthine, guanine, and guanosine are effective precursors of guanosine triphosphate. Hypoxanthine, inosine, and adenosine may be converted to both adenosine triphosphate and guanosine triphosphate.

The inhibition, 6-diazo-5-oxo-L-norleucine, interferes with the utilization of xanthine for guanosine triphosphate formation but does not inhibit the utilization of guanine.

In contrast to the findings in vitro, the administration of adenine, in vivo, results in equal labeling of the purine portions of both adenosine triphosphate and guanosine triphosphate.

**REFERENCES**
