Pyrroline-5-carboxylate Stimulates the Conversion of Purine Antimetabolites to Their Nucleotide Forms by a Redox-dependent Mechanism*

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The activation of purine antimetabolites to their respective nucleotides is a step critical to their effectiveness as chemotherapeutic agents. Erythrocytes, with their relatively simple purine metabolism, are useful as a model for identifying mechanisms which enhance this 5-phosphoribosyl 1-pyrophosphate (P-Rib-PP)-dependent activation. We previously showed that pyrroline-5-carboxylate, a physiologic intermediate in the interconversions of proline, ornithine, and glutamate, markedly stimulated the pentose phosphate pathway, increased the formation of P-Rib-PP, and increased purine incorporation into nucleotides. We now report that the events initiated by pyrroline-5-carboxylate markedly increased the activation of 6-thiopurine, 6-thioguanine, and azathioprine to their respective nucleotides in intact human erythrocytes. The mechanism of this effect was directly demonstrated in studies using the conversion of hypoxanthine to inosine monophosphate as a model for pyrroline-5-carboxylate-mediated stimulation of P-Rib-PP-dependent nucleotide formation. Since the P-Rib-PP-dependent activation of these chemotherapeutic agents may be important to their clinical effectiveness, the events initiated by pyrroline-5-carboxylate may provide new insight into the nature of tumor sensitivity and resistance to these agents.

Mature human erythrocytes readily incorporate preformed purines into nucleotides by the salvage pathway, but they cannot synthesize nucleotides de novo (1, 2). With their relatively simple system for purine metabolism, erythrocytes provide an experimental model for defining the regulation of purine uptake, processing, and release. Their metabolic features make them especially useful in pharmacologic investigations to elucidate mechanisms for activation of purine antimetabolites to their respective nucleotides (3, 4), an activation which is mediated by hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8).

The formation of nucleotides by hypoxanthine-guanine phosphoribosyltransferase in intact erythrocytes depends primarily on the availability of P-Rib-PP (5), which is synthesized from Rib-5-P and ATP by P-Rib-PP synthetase (Ribosephosphate pyrophosphokinase, EC 2.7.6.1). P-Rib-PP synthetase is regulated by many factors, but the cellular production of P-Rib-PP in vitro appears to be absolutely dependent on medium phosphate at supraphysiologic concentrations (6, 7). Although increased availability of Rib-5-P may also affect P-Rib-PP synthesis (8), the regulated formation of Rib-5-P for P-Rib-PP and nucleotide production has not been emphasized. To our knowledge, the augmentation of nucleotide production from purines and purine analogues by a naturally occurring intermediate has not been described.

We previously showed that 14C-1-oxo-pyrroline-5-carboxylate, a physiologic intermediate in the interconversions of proline, ornithine, and glutamate, markedly stimulated the pentose phosphate pathway (9-11), increased the formation of P-Rib-PP, and increased the incorporation of 14C-labeled purines into nucleotides (12). The stimulation is mediated by pyrroline-5-carboxylate reductase (EC 1.5.1.2) (13) which stoichiometrically oxidizes NADPH concomitant to the conversion of pyrroline-5-carboxylate to proline (14). Presumably, the increase in NADP+/NADPH ratios augments the production of Rib-5-P by the pentose phosphate pathway and thereby increases the production of P-Rib-PP.

We now report that the sequence of events initiated by pyrroline-5-carboxylate markedly increased the activation of purine antimetabolites, 6-thiopurine, 6-thioguanine, and azathioprine, to their respective nucleotides. The mechanism of this effect was directly demonstrated in studies using the conversion of hypoxanthine to IMP as a model for the stimulation of P-Rib-PP-dependent nucleotide formation by pyrroline-5-carboxylate. Since the P-Rib-PP-dependent activation of purine antimetabolites may be the limiting factor in the efficacy of these agents, the mechanism we have defined may be important not only as an adjunct to chemotherapy but also as an approach for understanding the nature of tumor sensitivity and resistance to these agents.

**EXPERIMENTAL PROCEDURES**

**Blood Preparation**—We obtained human venous blood by venipuncture from nonfasting normal adults, anticoagulated it with heparin (1000 units/ml, 0.1 ml/5 ml of blood), and separated erythrocytes from plasma and leukocytes by standard techniques (15). Erythrocytes were washed three times with normal saline and used for the experiments within 2 h of isolation.

**Purine Nucleotide Formation**—Erythrocytes (50 μl) were preincubated for 30 min at 37 °C without glucose or pyrroline-5-carboxylate in medium containing 145 mM NaCl, 10 mM Tris-HCl, pH 7.4, 0.5, 10, and 25 mM sodium phosphate, pH 7.4, 1.2 mM MgCl₂, in a total volume of 1 ml. After preincubation, 25 μM [8-14C]hypoxanthine, 2.5 μM glucose, differentially labeled with 14C in some experiments ([1-14C]glucose or [6-14C]glucose) were added to all the preparations. 1-Pyrroline-5-carboxylate enzymatically synthesized and purified by
Effect of Pyrroline-5-carboxylate on Purine Nucleotides

RESULTS

Pyrroline-5-carboxylate Stimulates the Conversion of Purine Antimetabolites to Their Respective Nucleotides—In intact human erythrocytes incubated with 6-thiopurine, pyrroline-5-carboxylate markedly increased the production of 6-thiopurine nucleotides (Table I). The amount of 6-thio-IMP in both control and treated cells increased with increasing duration of incubation (data not shown). The addition of pyrroline-5-carboxylate (0.5 mM) to preparations incubated for 60 min with 6-thiohypoxanthine (25 μM) increased the formation of 6-thio-IMP to 46 nmol/ml of cells as compared to 13 nmol/ml of cells in controls. The stimulation of nucleotide formation is dependent on the concentration of pyrroline-5-carboxylate. The formation of 6-thio-IMP increased with increasing concentration of pyrroline-5-carboxylate and reached a plateau at 0.5 mM (Fig. 1). The stimulatory effect of pyrroline-5-carboxylate on 6-thio-IMP production was observed at all concentrations of 6-thiohypoxanthine in intact human erythrocytes (Fig. 2). With 6-thiohypoxanthine at a saturating concentration of 50 μM, pyrroline-5-carboxylate increased 6-thio-IMP production from 25 to 145 nmol/h/ml of cells. Thus, pyrroline-5-carboxylate increased the capacity of erythrocytes to convert 6-thiohypoxanthine to 6-thio-IMP.

To further document the effect of pyrroline-5-carboxylate on the formation of 6-thio-IMP, we first generalized the effect to erythrocytes from five normal adults (three males, two females, age 30–43 years) (Table I). After 60 min of incubation with 6-thiohypoxanthine (25 μM), incubated control cells had 6-thio-IMP levels of 23.4 ± 5.31 (mean ± S.E.) nmol/ml of cells whereas the levels in cells incubated with pyrroline-5-carboxylate (0.5 mM) were 88.3 ± 12.84 nmol/ml of cells (p < 0.01). Thus, the effect on the formation of 6-thio-IMP was a consistent finding in erythrocytes from all subjects in our normal population. The effect of pyrroline-5-carboxylate on the metabolism of antimetabolites was not limited to 6-thiohypoxanthine but could be extended to the metabolism of other purine antimetabolites, e.g., 6-thioguanine and azathioprine. Erythrocytes from the same population of normal adults used for the studies on 6-thiohypoxanthine were incubated with the respective antimetabolite at a concentration of 25 μM. We found that pyrroline-5-carboxylate added at a concentration of 0.5 mM markedly increased the incorporation of all three purine antimetabolites to their respective nucleotides (Table I). Thus, this effect could be generalized to several purine antimetabolites which apparently are converted to their respective nucleotides by a common mechanism.

The Effect of Pyrroline-5-carboxylate on the Formation of Physiologic Nucleotides—Although we previously hypothesized that pyrroline-5-carboxylate initiates a redox-dependent cascade resulting in increased P-Rib-PP and nucleotides (12), additional studies are necessary to define the mechanism stimulating the activation of purine antimetabolites. The conversion of hypoxanthine to IMP serves as a model for elucidating this mechanism since the conversion of these antimetabolites to their respective mononucleotides is mediated by hypoxanthine-guanine phosphoribosyltransferase. We chose a physiologic precursor to avoid possible deleterious effects introduced by antimetabolites. In intact human erythrocytes incubated with hypoxanthine, pyrroline-5-carboxylate markedly increased the net production of IMP. After 90 min of incubation with hypoxanthine and pyrroline-5-carboxylate, the IMP pool was 527 nmol/ml of cells as compared to a pool of 72 nmol/ml of cells in freshly isolated erythrocytes. Thus, the IMP pool can become a large component of total purine nucleotides whereas in erythrocytes isolated from human
Effect of Pyrroline-5-carboxylate on Purine Nucleotides

The effect of pyrroline-5-carboxylate on the 6-thio-IMP pool versus 6-thioguanine. Freshly isolated erythrocytes were incubated as described under “Experimental Procedures.” 25 μM 6-thioguanine, 2.5 mM glucose, and pyrroline-5-carboxylate at various concentrations were added to the medium. The duration of incubation was 60 min. Cells were spun down in a microfuge centrifuge and the pellet taken up in 200 μl of 12% trichloroacetic acid with 2 mM dithiothreitol. After an additional centrifugation nucleotides in the supernatant were analyzed by high pressure liquid chromatography. 6-Thio-IMP was in the effluent and monitored by obtaining the absorbance at 320 nm and the amount was calculated by the data module with the use of external standards.

Fig. 2 (center). The effect of pyrroline-5-carboxylate on the 6-thio-IMP pool versus 6-thioguanine. The incubation conditions are as described under “Experimental Procedures” and in the legend for Fig. 1. The concentration of pyrroline-5-carboxylate was 0.5 mM and the duration of incubation was 60 min. Values for treated ( – ) and control ( – ) cells are shown.

Fig. 3 (right). The effect of pyrroline-5-carboxylate on IMP formation in intact human erythrocytes. Freshly isolated erythrocytes were incubated as described under “Experimental Procedures.” 25 μM [8-14C] hypoxanthine, 2.5 mM glucose, and 0.5 mM pyrroline-5-carboxylate where indicated were added to the medium and the cells harvested at the indicated times. Cells were spun down in a microfuge centrifuge and the pellet taken up in 1 ml of 12% trichloroacetic acid, vortexed, and recentrifuged. Nucleotides in the supernatant were analyzed by high pressure liquid chromatography. The amount of IMP was measured by obtaining the absorbance at 254 nm and the incorporation of label into IMP was quantitated by measuring the radioactivity in 1-ml fractions of column effluent. The total IMP in treated ( – ) and control ( – ) cells as well as the incorporation of hypoxanthine into IMP in pyrroline-5-carboxylate-treated ( – ) and control ( – ) cells are shown.

Table I

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>IMPnmol/ml of cells</th>
<th>Adenine nucleotides nmol/ml of cells</th>
<th>Total IMP + adenine nucleotides nmol/ml of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>111 ± 7</td>
<td>1294 ± 55</td>
<td>1405 ± 49</td>
</tr>
<tr>
<td>60</td>
<td>326 ± 28</td>
<td>1324 ± 76</td>
<td>1650 ± 67</td>
</tr>
</tbody>
</table>

*p < 0.001 NS, not significant.

Table II

The effect of pyrroline-5-carboxylate on IMP formation in intact human erythrocytes. Erythrocytes from normal volunteers were incubated as described in the legend for Fig. 1. The concentration of pyrroline-5-carboxylate was 0.5 mM and the duration of incubation was 60 min. The values shown represent mean ± S.E. of at least three determinations.

Table III

The effect of pyrroline-5-carboxylate on the incorporation of purine antimetabolites into nucleotides

Erythrocytes from normal volunteers were incubated as described in the legend for Fig. 1. The duration of incubation with pyrroline-5-carboxylate was 60 min. The concentration of 6-thioguanine, azathioprine, or 6-thioguanine was 25 μM and pyrroline-5-carboxylate was 0.5 mM. The values shown represent mean ± S.E. of at least three determinations.

Table IV

The effect of pyrroline-5-carboxylate on purine nucleotide pools

Erythrocytes from normal volunteers were incubated as described in the legend for Fig. 1. The duration of incubation with pyrroline-5-carboxylate was 0.5 mM and pyrroline-5-carboxylate was 0.5 mM. The values shown represent mean ± S.E. of six determinations.

References

1. Pyrroline-5-carboxylate

2. Pyrroline-5-carboxylate

3. Pyrroline-5-carboxylate

4. Pyrroline-5-carboxylate

5. Pyrroline-5-carboxylate
In order to characterize this pyrroline-5-carboxylate-mediated increase in net production of IMP, we measured the incorporation of \([\text{\textsuperscript{14}C}]\)hypoxanthine into nucleotides. As shown in Fig. 3, pyrroline-5-carboxylate markedly stimulated the formation of \([\text{\textsuperscript{14}C}]\)IMP from \([\text{\textsuperscript{14}C}]\)hypoxanthine. In agreement with other published studies in human erythrocytes, IMP was the only labeled nucleotide with \([\text{\textsuperscript{14}C}]\)hypoxanthine as precursor (19). Our studies using cells from six normal subjects showed that the incorporation of \([\text{\textsuperscript{14}C}]\)hypoxanthine into \([\text{\textsuperscript{14}C}]\)IMP was 233 ± 19 nmol/l/hr of cells in treated cells and 78 ± 6 nmol/l/hr of cells in controls. These values closely reflect the increase in the IMP pool size and support the interpretation that it is an increase in the incorporation of exogenous hypoxanthine rather than the endogenous turnover of adenosine which is the basis for the pyrroline-5-carboxylate-mediated increase in IMP pools.

The Effect of Pyrroline-5-carboxylate and Phosphate on P-Rib-PP and IMP Formation—We previously reported that pyrroline-5-carboxylate markedly increased P-Rib-PP formation in intact red cells (12). However these studies as well as the aforementioned studies on IMP formation were performed at saturating concentrations of pyrroline-5-carboxylate in order to maximize the effect. Importantly, the effects on both P-Rib-PP and IMP pools were observed at much lower concentrations of pyrroline-5-carboxylate. Even with pyrroline-5-carboxylate concentrations of \(5 \times 10^{-5} \text{ M}\), the levels of P-Rib-PP and IMP were markedly increased (data not shown). The magnitude of the effect on both P-Rib-PP and IMP increased with increasing pyrroline-5-carboxylate concentrations. However, with pyrroline-5-carboxylate concentrations greater than \(1.5 \times 10^{-4} \text{ M}\), IMP reached a plateau whereas P-Rib-PP continued to increase. Presumably, IMP did not increase further because hypoxanthine-guanine phosphoribosyltransferase was saturated relative to P-Rib-PP (20).

For intact red cells, the dependence of both P-Rib-PP synthesis and nucleotide formation on supraphysiologic concentrations of phosphate has been reported by a number of investigators (5-7). Consequently, we used an incubation medium containing phosphate at an optimal concentration of 25 mM so that the phosphate-dependent activity of P-Rib-PP synthetase was not a limiting factor. Both IMP formation as measured by the incorporation of \([\text{\textsuperscript{14}C}]\)-labeled hypoxanthine and P-Rib-PP production were stimulated by pyrroline-5-carboxylate at lower phosphate concentrations, but the magnitude of the effect was considerably less (Fig. 4). The stimulatory effect of pyrroline-5-carboxylate was superimposed on that of the phosphate effect at all phosphate concentrations necessary to produce a phosphate-dependent effect. Importantly, even at phosphate concentrations which were saturating, pyrroline-5-carboxylate produced a marked increase in P-Rib-PP and IMP formation over that of phosphate controls.

The Effect of Pyrroline-5-carboxylate Is Due to Activation of the Oxidative Branch for Glucose Metabolism—The previously demonstrated stimulation of the pentose shunt by pyrroline-5-carboxylate (9-12) is the likely mechanism for the effect on nucleotide formation from hypoxanthine and antimetabolites. According to this hypothesis, increased production of Rib-5-P and P-Rib-PP by the oxidative branch for glucose metabolism (pentose shunt) would augment the incorporation of hypoxanthine by hypoxanthine-guanine phosphoribosyltransferase. However, Rib-5-P can also originate from glucose metabolized in the nonoxidative branch of the pentose phosphate pathway by rearrangement of fructose-6-phosphate and glyceraldehyde-3-phosphate in reactions catalyzed by transketolase and transaldolase. Therefore, we sought direct evidence that the increased net synthesis of nucleotides was due to the redox-dependent activation of the oxidative branch by pyrroline-5-carboxylate. We incubated erythrocytes with glucose differentially labeled in the C-1- or C-6-positions and measured the incorporation of labeled ribose into IMP in the presence of hypoxanthine. The rationale for these experiments is based on the fact that glucose is decarboxylated at the 1-position in the oxidative branch (21). Consequently, ribose in IMP labeled with \([\text{\textsuperscript{13}C}]\)glucose can originate only in the nonoxidative branch whereas that labeled with \([6,\text{\textsuperscript{14}C}]\)glucose can originate from both.

We found that essentially all of the ribose incorporated into IMP with pyrroline-5-carboxylate stimulation was produced by the oxidative branch. In control incubations, 79% of the ribose in IMP originated from nonoxidative metabolism. With pyrroline-5-carboxylate stimulation the nonoxidative branch did not increase its contribution to IMP in spite of a 400% increase in the IMP pool (Table III). In fact, the IMP formed under pyrroline-5-carboxylate stimulation contained ribose which was derived almost entirely from the oxidative branch.

**TABLE III**

The incorporation of differentially labeled glucose into inosine monophosphate

<table>
<thead>
<tr>
<th></th>
<th>C-1 labeled</th>
<th>C-6 labeled</th>
<th>IMP pool</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm/m(\mu)l</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cells</td>
<td>mmol/ml cells</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.73 (\times) (10^6)</td>
<td>72</td>
<td>0.98 (\times) (10^6)</td>
</tr>
<tr>
<td>Pyrroline-5-carboxylate</td>
<td>1.19 (\times) (10^6)</td>
<td>117</td>
<td>3.80 (\times) (10^6)</td>
</tr>
</tbody>
</table>

**Fig. 4.** The effect of pyrroline-5-carboxylate on P-Rib-PP and IMP pools versus phosphate concentration. The incubation conditions are as described under "Experimental Procedures" and in the legend for Fig. 3. P-Rib-PP in treated (\(\triangle\) - - -\(\triangle\)) and control (\(\bullet\) ---\(\bullet\)) cells and total IMP in treated (\(\Diamond\) - - -\(\Diamond\)) and control (\(\bigcirc\) ---\(\bigcirc\)) cells are shown.
Effect of Pyrroline-5-carboxylate on Purine Nucleotides

Antitumor activity of the purine and pyrimidine analogs used for cancer chemotherapy requires that they be enzymatically converted in situ to their respective monophosphates. Hypoxanthine-guanine phosphoribosyltransferase activates 6-thioguanine, 6-thioguanine, and azathioprine, and orotate phosphoribosyltransferase activates 5-fluorouracil. Since both enzymes require P-Rib-PP, the regulation of P-Rib-PP levels may be critically important for drug efficacy. An increase in P-Rib-PP levels, thereby augmenting the activation of antimetabolites, has been proposed as the basis underlying the clinical effectiveness of certain combination drug regimens. For example, methotrexate given prior to 5-fluorouracil increases the incorporation of 5-fluorouracil into nucleotides by increasing the intracellular content of P-Rib-PP. The increase in P-Rib-PP is due to the inhibition by methotrexate of P-Rib-PP utilization for de novo purine synthesis (22).

The finding that pyrroline-5-carboxylate increases P-Rib-PP levels, thereby augmenting the activation of purine antimetabolites, provides a new approach for modifying the clinical effectiveness of purine and pyrimidine antimetabolites in cancer chemotherapy. Insight into the regulated production and metabolism of pyrroline-5-carboxylate by various tumors may also be relevant in understanding tumor sensitivity and resistance to these chemotherapeutic agents. Human erythrocytes were chosen for this initial demonstration because of their relatively simple metabolic system for purines allowing for unambiguous demonstration of mechanisms. However, the assessment of possible clinical applications requires the extension of these observations to cultured cells. Cultured cells do respond to pyrroline-5-carboxylate by increasing their pentose phosphate pathway activity (10, 11) and, in fact, also respond to proline and ornithine, physiological precursors of pyrroline-5-carboxylate. The possibility that they respond in a manner similar to erythrocytes in the activation of antimetabolites is under active investigation.

Our studies using the conversion of hypoxanthine to IMP to define the mechanism of the pyrroline-5-carboxylate effect provide strong support for our hypothesis that pyrroline-5-carboxylate initiates a sequence of events resulting in increased production of nucleotides. This sequence includes 1) the oxidation of NADPH by pyrroline-5-carboxylate reducase accompanying the conversion of pyrroline-5-carboxylate to proline, 2) increased oxidation of glucose via the pentose phosphate pathway due to the altered redox state, 3) increased P-Rib-PP formation due to increased production of Rib-5-P, and 4) increased incorporation of purines in the P-Rib-PP-dependent salvage pathway.

In our studies as well as those previously reported (5–7), supraphysiologic phosphate concentrations were necessary to demonstrate the production of P-Rib-PP and nucleotides in red cells. The phosphate-dependent activation of P-Rib-PP synthetase is the apparent reason for this necessity. We observed that pyrroline-5-carboxylate increased P-Rib-PP and nucleotide production at all concentrations of phosphate necessary for P-Rib-PP production. More importantly even at concentrations of phosphate where P-Rib-PP levels had plateaued, pyrroline-5-carboxylate produced a marked increase in both P-Rib-PP and nucleotide formation over that of phosphate alone. Not only does this finding suggest that pyrroline-5-carboxylate works via a mechanism distinct from that of phosphate but also that phosphate is a necessary but not sufficient mechanism for maximum P-Rib-PP and nucleotide formation. Although the tissue site(s) with phosphate levels adequate to support the in vivo incorporation of nucleotides in red cells has not yet been identified, existence of these local sites may exist especially in tissues with high turnover of ATP (23). Alternatively, increases in intracellular phosphate by altered metabolic states may provide the conditions necessary for the demonstrated rapid turnover of certain red cell nucleotides (IMP) in vivo. The complex relationships among pyrroline-5-carboxylate, phosphate, and glucose concentrations in regulating P-Rib-PP and nucleotides have been investigated in our laboratory and will be the subject of a subsequent report.

Our findings clearly show that the pyrroline-5-carboxylate effect is mediated by the stimulation of Rib-5-P production by the oxidative branch of the pentose phosphate pathway. However, the relative flow into nucleotides from the oxidative and nonoxidative branches of the pentose phosphate pathway remains a topic of debate. The downgrading of the contribution made by the oxidative branch is based on reports that erythrocytes and cultured fibroblasts from patients with glucose-6-phosphate dehydrogenase deficiency have normal levels of P-Rib-PP and nucleotides (7, 24). Of course the deficiency is frequently incomplete, but even in patients with undetectable glucose-6-phosphate dehydrogenase activity, levels of P-Rib-PP similar to that of normal cells have been reported (7). In addition, studies in tissues with differentially labeled glucose suggest that the nonoxidative branch predominates (25). However, under conditions of carbohydrate limitation, cultured cells derive their complement of nucleotide pentose almost entirely from the oxidative branch (26). Our findings show that although the nonoxidative branch supplies the majority of the nucleotide pentose under control conditions, it is the oxidative branch which provides the pentose with pyrroline-5-carboxylate stimulation. Thus, a pattern emerges which may in part resolve the controversy. Although either branch can adequately supply pentose under specific experimental conditions, it is the oxidative branch which can be recruited to supply the pentose when the demand is maximal. Of course, additional studies are required to establish this correlation.

The pyrroline-5-carboxylate-stimulated activation of purine antimetabolites have clear pharmacologic implications. Moreover, the finding that pyrroline-5-carboxylate produced a marked increase in the net production of IMP may be physiologically important. Others have proposed that in erythrocytes IMP may serve as a "mobile pool" (19), i.e. a pool of nucleotides (IMP). However, under conditions of carbohydrate limitation, cultured cells will be able to supply the pentose when the demand is maximal. Of course, additional studies are required to establish this correlation.

Although pyrroline-5-carboxylate markedly stimulated the net production of nucleotides by a redox-dependent mechanism in vitro, the physiologic linkage between this intermediate of amino acid metabolism and redox-dependent pathways in vivo has not been established. Nevertheless, in cultured cells not only pyrroline-5-carboxylate but also its precursors, proline and ornithine, can stimulate the pentose phosphate pathway (11). The mechanism of this effect in cultured cells has been related to the cycling of pyrroline-5-carboxylate and proline in an in vitro reconstituted system (29). Although erythrocytes cannot produce pyrroline-5-carboxylate endogenously, they readily take up pyrroline-5-carboxylate released from other cells, e.g. hepatocytes, and rapidly convert pyrroline-5-carboxylate to proline (30, 31). In
fact, our previous studies show that any pyrroline-5-carboxylate released by tissues would be cleared from plasma by erythrocytes. The regulation of pyrroline-5-carboxylate production and release from specific tissues remains an important and interesting question.

Whatever the physiologic or pathophysiologic role of this regulation of purine nucleotide pools in erythrocytes, the phenomenon described in this report may have clinical implications. Presumably, the mechanism defined in these studies when extended to tumor cells may provide adjunctive approaches for cancer chemotherapy. Furthermore, erythrocytes loaded with purine antimetabolites may serve as a carrier system to deliver and release these agents either as the base or as the respective nucleoside to the specific tumor site (32). Finally, IMP-loaded red cells may be useful in promoting post-traumatic healing processes, e.g. the replenishing of myocardial purine nucleotides following infarction (33).

REFERENCES
Pyroline-5-carboxylate stimulates the conversion of purine antimetabolites to their nucleotide forms by a redox-dependent mechanism.

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