Regulation of 5-Phosphoribosyl 1-Pyrophosphate and of Hypoxanthine Uptake and Release in Human Erythrocytes by Oxypurine Cycling*

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Uptake and release of purines by red blood cells has been shown to be markedly sensitive to changes in pH, inorganic phosphate (Pi), and oxygen concentration (Berman, P., Black, D., Human, L., and Harley, E. (1988) J. Clin. Invest. 82, 980–986). The mechanism of this regulation has been further studied. We have shown that incubation of red cells in medium containing xanthine oxidase rapidly and completely depletes intracellular hypoxanthine and causes accumulation of 5-phosphoribosyl 1-pyrophosphate (PRPP) at physiological Pi concentrations. Hypoxanthine release from intracellular IMP is strictly dependent on PRPP deprecation, induced by either alkalinizing the cells or by adding excess adenine. Xanthine oxidase abolishes this dependence. Oxygen depletion enhances adenine uptake and prevents hypoxanthine release. The results suggest that hypoxanthine release is governed by PRPP-dependent recycling of hypoxanthine to IMP.

We propose that PRPP accumulation in red cells is regulated by a substrate cycle, comprising hypoxanthine, IMP, and inosine. Cycle flux is controlled by Pi inhibition and 2,3-bisphosphoglycerate activation of purine-5'-nucleotidase, which converts IMP to inosine. Oxypurine cycling may account for the sensitive control of purine uptake and release by changes in pH and oxygen tension that occur physiologically.

Enzymes of purine metabolism are present in human erythrocytes at high specific activity (1). Since mammalian red cells are anucleate and contain neither DNA nor RNA, the role of these enzymes is not immediately apparent. It has been suggested that they are implicated in the transport of purines across the membrane, utilizing a specific carrier (7). Phosphorylation of these bases, and the pyrimidine base orotate, is dependent on 5-phosphoribosyl 1-pyrophosphate (PRPP), as substrate for the appropriate phosphoribosyl transferase (8). This reaction enables cells to take up and concentrate purines in the form of nucleotides.

Synthesis of PRPP from ribose 5-phosphate and ATP is catalyzed by 5-phosphoribosyl 1-pyrophosphate synthetase. The enzyme has an absolute requirement for Pi for its activation, with a $K_{m}$ of approximately 1 mM (9). A major difference between nucleated cells and mammalian erythrocytes is the Pi dependence of PRPP accumulation. Nucleated cells synthesize PRPP maximally at physiological [Pi] (=1 mM), whereas red cells require up to 80 mM Pi to achieve similar levels of PRPP (10). The low levels of PRPP (1-5 x 10^{-6} M) found in normal red cells, or cells incubated at physiological [Pi] (=1 mM) (8), have been attributed to the potent inhibition exerted on the enzyme by ADP and 2,3-bisphosphoglycerate (2,3-BPG), which is relieved by superphysiological concentrations of Pi, (11). The concentration of ribose 5-phosphate in red cells is well below the $K_{m}$ for PRPP synthetase, estimated at 33 x 10^{-6} M (2, 8). However, increases in ribose 5-phosphate by incubation with methylene blue (11), pyrroline-5-carboxylate (12), or inosine (11), to levels above the $K_{m}$ for PRPP synthetase, have little effect on PRPP accumulation at low [Pi]. Pi concentrations greater than 10 mM are required before increased steady state levels of PRPP are observed.

Adenine nucleotide catabolism in erythrocytes has been studied by Bontemps et al. (4), who showed that an increase in pH from 7.4 to 7.7 caused a 20-fold enhancement of hypoxanthine efflux. This efflux was inhibited up to 90% by 10 mM Pi. Whelan and Bagnara (5) showed that, while IMP dephosphorylation was markedly enhanced by oxygen and inhibited by Pi, ATP catabolism was unaffected by such manipulations.

We have previously described the effects of pH, Pi, and oxygen on red cell hypoxanthine uptake and release (13). Enhanced uptake was observed with high [Pi], low pH, and low pO$_{2}$. The effect of oxygen was ascribed to the direct relationship between red cell oxygenation and intracellular levels of free 2,3-BPG, since oxygen displaces 2,3-BPG bound to deoxygenated hemoglobin (14). 2,3-BPG is an inhibitor of PRPP synthetase (11) and an activator of purine-5'-nucleotidase (15).

Substrate cycles that regulate metabolic flux have been described by Newsome (16). An example is the two-component cycle, comprising the glycolytic intermediates, fructose...
6-phosphate and fructose 1,6-bisphosphate. This cycle permits sensitive control of glucose uptake and release in liver tissue by allosteric effectors of phosphofructokinase activity, including ATP, AMP, P_{i}, and fructose 2,6-bisphosphate.

In this study, we show that flux through a three-component oxypurine cycle, comprising hypoxanthine, IMP, and inosine, regulates PRPP accumulation and the release of nucleotides from human erythrocytes. A diagram of the oxypurine cycle and its metabolic inter-relationships is presented in Fig. 1.

**EXPERIMENTAL PROCEDURES**

**Materials**-[8-14C]Hypoxanthine, [8-14C]adenine, [8-14C]guanine, [6-14C]orotate, and [8-14C]adenosine (all 57 Ci/mol) were purchased from Amersham Corp., Amersham, United Kingdom. Xanthine oxidase was obtained from Boehringer Mannheim GmbH, Federal Republic of Germany. Dibutyl phthalate was purchased from F. Merck, Darmstadt, F.R.G.; PRPP, superoxide dismutase, and Ficoll-Histopaque from Sigma; and DEAE-Spectra/Gel M from Spectrum Medical Industries, Los Angeles, CA.

**Preparation of Erythrocytes**—Heparinized whole blood was obtained from healthy volunteers, mixed with 5% dextran in 0.15 M NaCl at a ratio of 4:1 (v/v), and allowed to sediment for 90 min. The upper layer containing granulocytes and platelets was discarded, and the dextran sedimentation step was repeated. The red cells were washed once with saline, layered onto Ficoll-Histopaque, and centrifuged to remove contaminating mononuclear cells. The red cell pellet was washed three more times with saline to ensure complete removal of leukocytes and platelets. For some experiments, a simplified procedure was adopted, whereby whole blood was centrifuged, the plasma and buffy coat were discarded, and the red cells were washed once with 0.15 M NaCl. Either method yielded comparable results.

**Incubation of Erythrocytes**—Washed erythrocytes were incubated at 37 °C in medium consisting of 50 mM Hepes, 120 mM NaCl, and 5 mM glucose at a cell to medium ratio of 1:2 (v/v). pH was adjusted with NaOH and measured after the addition of cells on an ABL blood gas analyzer (Radiometer, Copenhagen, Denmark). This is important, as buffering by hemoglobin causes significant alterations in pH. P_{i} concentration of the medium was varied by addition of NaH_{2}PO_{4}. To obtain quantitative conversion of [14C]hypoxanthine and [14C]adenine to labeled IMP and adenylyl nucleotides, respectively, cells were incubated at pH 7.0 in 5-10 mM P_{i} with the appropriate [14C]purine base, and, because >98% depleted of the label, xanthine oxidase and superoxide dismutase were used at final concentrations of 0.8 and 2.5 x 10^{-3} units/ml, respectively.

**Quantitation of Radiolabeled Purine Species**—Aliquots of cell suspension, typically 50-100 μl, were centrifuged through dibutyl phthalate for 30 s at 10,000 g in a 1.5-ml Microfuge tube, as described by Wohlhueter et al. (17), and then washed two more times with saline, layered onto Ficoll-Histopaque, and centrifuged to remove an equal volume of 0.6 M perchloric acid. The dibutyl phthalate layer was discarded, and the red cell pellet was resuspended in an equal volume of 0.15 M NaCl, followed by the addition of 2 volumes of 0.6 M perchloric acid. Denatured protein was removed by centrifugation, and the supernatant medium was deproteinized with 0.15 M perchloric acid.

**PRPP Assay**—PRPP was measured by the formation of [14C]IMP in the presence of excess [14C]hypoxanthine and a crude hemolysate as the source of hypoxanthine-guanine phosphoribosyltransferase, using a modification of the method previously described (13). Red cell suspension, typically 100 μl, was mixed with 10 μl of 10 mM EDTA to stabilize PRPP and heated at 100 °C for 3 min. Denatured protein was removed by centrifugation at 10,000 g for 30 s, and 50 μl of supernatant was mixed with 12.5 μl of 10% (w/v) activated charcoal, to absorb purine nucleotides and bases. The charcoal was removed by centrifugation, and 30 μl of supernatant was mixed with 15 μl of [14C]hypoxanthine-phosphoribosyltransferase. After incubation at 37 °C for 1 h, 19 μl of 1.5 M perchloric acid was added. The denatured protein was removed by centrifugation, and 50 μl of supernatant was neutralized with 3 μl of 2.5 M K$_2$CO$_3$. The K$_2$CO$_3$ precipitate was removed by centrifugation, and the supernatant was applied to an anion exchange HPLC column, to quantitate the [14C]IMP formed. The mean recovery obtained for aqueous solutions of commercially available PRPP in the concentration range of 0-60 μM was 60%.

**Purine 5'-Nucleotidase Activity**—The purine-specific 5'-nucleotidase from human erythrocytes was partially purified by the methods of Bontemps et al. (15), up to and including DEAE-Trisacryl chromatography. Eluates containing enzyme activity were pooled and incubated at 55 °C with 50 mM Mes, pH 6.3, 10 mM MgCl$_2$, 200 μM [14C]hypoxanthine, 57 Ci/mol, and 40% (w/v) of a hemolysate, prepared as described by Steyn (18), as the source of hypoxanthine-guanine phosphoribosyltransferase. After incubation at 37 °C for 1 h, 19 μl of 1.5 M perchloric acid was added. The denatured protein was removed by centrifugation, and 50 μl of supernatant was neutralized with 3 μl of 2.5 M K$_2$CO$_3$. The K$_2$CO$_3$ precipitate was removed by centrifugation, and the supernatant was applied to an anion exchange HPLC column, to quantitate the [14C]IMP formed. The mean recovery obtained for aqueous solutions of commercially available PRPP in the concentration range of 0-60 μM was 60%.

**Assay of Purine 5'-Nucleotidase Activity**—The purine-specific 5'-nucleotidase from human erythrocytes was partially purified by the methods of Bontemps et al. (15), up to and including DEAE-Trisacryl chromatography. Eluates containing enzyme activity were pooled and incubated at 55 °C with 50 mM Mes, pH 6.3, 10 mM MgCl$_2$, and 0.2 mM IMP in a final volume of 200 μl. Where appropriate, PRPP and Na$_2$HPO$_4$ were added at a final concentration of 1 mM. 50 μl aliquots of the reaction mixture were removed at hourly intervals and deproteinized with 0.6 M perchloric acid. The extract was neutralized with K$_2$CO$_3$, and its inosine content was quantitated by reverse phase HPLC, using external inosine standards.

**High Pressure Liquid Chromatography**—Purine nucleotides and purine bases were separated by anion exchange and reverse phase HPLC, respectively, using a Spectra-Physics A-901 liquid chromatograph operated with software for data manipulation. Anion exchange chromatography was performed on a Waters Associates 8P SAX 10 Radial Pak cartridge, housed in a radial compression module. A linear gradient was developed over 8 min from 45 mM HCO$_3$O$_4$, adjusted to pH 4.5 with H$_3$PO$_4$, to 1 M Na$_2$HPO$_4$, adjusted to pH 2.7 with HCO$_3$O$_4$, at a flow rate of 2 ml/min. The high concentration of perchlorate in the mobile phase was necessary to allow elution of ATP and GTP. Reverse phase chromatography was performed on a Vydac 201 H-5C 18-cartridge. The linear eluant was 1 mM KH$_2$PO$_4$ in water (solvent A), and the nonlinear eluant was acetonitrile, 25% (v/v), in solvent B (water). A linear gradient was developed over 10 min from 0-100% solvent B at a flow rate of 1.3 ml/min. Reverse phase separation of inosine, adenosine, guanosine, and guanine was monitored using a Packard Tri-Carb liquid scintillation spectrophotometer. [14C]Purine concentration was calculated from the specific activity and expressed in micromolar concentrations or nanomoles per ml of red blood cells for medium and cells, respectively.

**Extracts were neutralized with 6% (v/v) 2.5 M K$_2$CO$_3$, and 20 μl of supernatant was analyzed by HPLC.**

**Manipulation of Erythrocyte Oxygen Tension**—Oxygen tension (PO$_2$) was decreased or increased by bubbling halogen or air, respectively, through erythrocyte suspensions for 10-15 min. Frothing was minimized by applying silicone lubricant around the glass tube above the meniscus of the suspension. Suspensions were kept in stoppered syringes to prevent changes in PO$_2$, PO$_2$ measurements were performed on a Radiometer ABL blood gas analyzer.
RESULTS

The effect of xanthine oxidase on intracellular levels of hypoxanthine is shown in Fig. 2. Red cells were equilibrated in medium containing 20 μM \[^{14}C\]hypoxanthine, then xanthine oxidase was added. Changes in intra- and extracellular \[^{14}C\]purine concentrations were monitored. Xanthine oxidase caused immediate depletion of both medium and cellular \[^{14}C\]hypoxanthine, to levels below 1 μM. In the medium, more \[^{14}C\]urate accumulated than \[^{14}C\]hypoxanthine originally present, whereas in the cells, \[^{14}C\]hypoxanthine depletion was not immediately accompanied by a concomitant increase in \[^{14}C\]urate. This suggests that xanthine oxidase did not enter the cells, but, by irreversibly oxidizing medium hypoxanthine, established a concentration gradient which rapidly led to depletion of the intracellular hypoxanthine pool.

Since PRPP is consumed when hypoxanthine is salvaged by hypoxanthine-guanine phosphoribosyltransferase, the effect of depleting endogenous hypoxanthine on accumulation of PRPP was studied. The results are shown in Table I. In the absence of xanthine oxidase, PRPP accumulation showed an absolute dependence on both low pH and high P,

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<th>-Xanthine oxidase</th>
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<td>0.2</td>
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placed this dependence on pH and P, PRPP accumulated irrespective of pH and in the absence of added P. This suggests that in intact cells P, may be required, not only to activate PRPP synthesis, but also to inhibit PRPP consumption by hypoxanthine.

By causing PRPP accumulation, xanthine oxidase should enhance nucleotide synthesis from purine and pyrimidine nucleobases which depend on phosphoribosyltransferases for salvage. The effect of xanthine oxidase on the incorporation of \(^{14}\)C-labeled adenine, guanine, orotate, and adenosine into nucleotides is shown in Fig. 3. Erythrocytes were incubated in medium containing 50 μM \(^{14}\)C-labeled base or nucleoside, either with or without xanthine oxidase, and the incorporation of label into the corresponding nucleoside triphosphate, which accounted for >90% of labeled nucleotide, was measured. Xanthine oxidase enhanced nucleotide synthesis from adenine (A), guanine (G), and orotate (C). Salvage of adenosine (D), catalyzed by adenosine kinase, which utilizes ATP rather than PRPP as substrate, is more rapid than phosphoribosyltransferase-catalyzed salvage of adenine and is essentially unaffected by the presence of xanthine oxidase. The limited extent of ATP synthesis from adenosine is due to the rapid degradation of adenosine to inosine by adenosine deaminase (5).

We have shown that uptake of hypoxanthine into IMP is enhanced 2- to 3-fold by a decrease in oxygen tension below 7 kPa (13). The effect of oxygen on adenine uptake by red cells is shown in Fig. 4. Cells were incubated with 10 μM \(^{14}\)C adenine at low (4.8 kPa) or high (26 kPa) oxygen tension, and the disappearance of radioactivity from the medium was monitored. Uptake of adenine was enhanced under anaerobic conditions (Fig. 4A). A sample of cells was removed from both suspensions after 30 min, and the distribution of radioactivity in the adenine-containing species was analyzed. There was greater synthesis of adenyate nucleotide in cells incubated anaerobically (Fig. 4B), when compared to the aerobically incubated control (Fig. 4C).

Erythrocytes incubated at pH 7.0 in medium containing 10 mM P, take up and quantitatively convert hypoxanthine to IMP (13). Factors governing subsequent hypoxanthine release were studied. Cells were enriched with \(^{14}\)CIMP as described.
Oxypurine Cycling in Human Erythrocytes

Fig. 3. Effect of xanthine oxidase on nucleotide synthesis from adenine, guanine, orotate, and adenosine in intact erythrocytes. Erythrocytes were incubated at pH 7.1 in medium containing 1 mM Pi, with 50 μM [14C]adenine (A), [14C]guanine (B), [14C]orotate (C), or [14C]adenosine (D), either in the presence (solid symbols) or absence (open symbols) of xanthine oxidase. The intracellular concentration of the corresponding [14C]-labeled nucleotide triphosphate was determined at the times indicated. RBC, red blood cells.

under “Experimental Procedures,” then resuspended in fresh medium devoid of Pi. At pH 6.94, negligible [14C]hypoxanthine was released, and PRPP levels were maintained at approximately 100 nmol/ml of cells (Fig. 5A). Increasing the pH of the resuspended cells to 7.71 resulted in a steady decline of PRPP and release of [14C]hypoxanthine, which began only once PRPP depletion was complete (Fig. 5B). Inclusion of 0.5 mM adenine in a suspension incubated at pH 7.71 resulted in the immediate efflux of [14C]hypoxanthine, and in PRPP levels that were low ab initio (Fig. 5C). Inclusion of xanthine oxidase in a suspension incubated at pH 7.71 also resulted in the immediate release of [14C]hypoxanthine (detected as [14C]-urate), but in this case, release was not coupled to depletion of PRPP. Indeed, PRPP levels declined more slowly than in the absence of the enzyme (Fig. 5D).

Failure of cells containing high levels of PRPP to release hypoxanthine raises the possibility of direct inhibition of purine-5'-nucleotidase by PRPP. For this reason, the effect of 1 mM PiPRPP on partially purified 5'-nucleotidase was studied and is shown in Fig. 6. PRPP had no appreciable effect on enzyme activity, whereas Pi, at the same concentration, was clearly inhibitory, as has been previously reported (15).

The effect of other bases and nucleosides on the release of hypoxanthine was investigated. Cells were enriched with [14C]IMP as described, then resuspended in media containing purine or pyrimidine bases or nucleosides at a final concentration of 0.2 mM. Hypoxanthine and guanine, and the nucleosides, inosine, and guanosine, which are rapidly converted to hypoxanthine or guanine, caused release of [14C]hypoxanthine at the same rate as did adenine (Fig. 5C). The pyrimidine bases, cytosine and uracil, failed to elicit hypoxanthine release (data not shown). While hypoxanthine and guanine, like adenine, readily consume PRPP in phosphoribosyltransferase-catalyzed reactions, the pyrimidine bases are not salvaged by phosphoribosyltransferases and would not be expected to affect PRPP levels.

Fig. 4. Effect of oxygen on adenine uptake and adenylate nucleotide synthesis. Erythrocytes were suspended at pH 7.1 in medium containing 1 mM Pi and divided into two portions which were equilibrated with helium or air to achieve oxygen tensions of 4.8 (open symbols) and 26 (closed symbols) kPa, respectively. [14C]Adenine was added at a final concentration of 10 μM, and the suspensions were incubated at 37 °C in sealed syringes. The concentration of [14C]adenine in the medium was determined at timed intervals (A). The difference between duplicate estimations was on average 10% of the mean. Cells removed from both suspensions after a 30-min incubation were extracted and analyzed by anion exchange HPLC to show the nature and extent of labeled nucleotide accumulation at pO2 4.8 kPa (B) and pO2 26 kPa (C).

Fig. 5. Effect of pH, excess adenine, and xanthine oxidase on the release of hypoxanthine from IMP-enriched cells. Erythrocytes were enriched with [14C]IMP, then resuspended in Pi-free medium at pH 6.94 (A) or at pH 7.71 (B), (C), and (D). C and D contained in addition 0.5 mM unlabeled adenine and xanthine oxidase, respectively. The suspensions were incubated at 37 °C, and aliquots were removed at timed intervals for determination of medium [14C]hypoxanthine (closed circles), medium [14C]-urate (closed squares), and intracellular PRPP (open triangles). RBC, red blood cells.
Fig. 6. Effect of PRPP on purine 5'-nucleotidase. Partially purified purine-specific 5'-nucleotidase was incubated with 0.2 mM IMP at 37 °C. Aliquots of the reaction mixture were removed at timed intervals, and the inosine formed was measured by reverse phase HPLC (open circles). In parallel incubations, PRPP (solid circles) or Pi (solid triangles) was included at a final concentration of 1 mM.

Fig. 7. Relationship between medium adenine concentration and hypoxanthine release from IMP-enriched cells. Cells were enriched with [14C]IMP as described under "Experimental Procedures." The suspension was divided into aliquots, and unlabeled adenine was added at a final concentration ranging from 0–130 μM. After a 10-min incubation, the medium [14C]hypoxanthine concentration (open symbols) and total intracellular [14C]purine concentration (closed circles) were measured. The average difference between duplicate assays of medium and cellular radioactivity was 2.8% and 0.7% of the mean, respectively. RBC, red blood cells.

The dependence of hypoxanthine release on medium adenine concentration is shown in Fig. 7. Cells enriched with [14C]IMP were incubated with varying concentrations of unlabeled adenine. Labeled hypoxanthine appearing in the medium and residual intracellular radioactivity was determined. A discontinuous relationship between adenine concentration and hypoxanthine release was observed. Negligible release of [14C]hypoxanthine occurred at medium adenine concentrations lower than 20 μM. Between 20 and 40 μM adenine, [14C]hypoxanthine efflux increased rapidly, while at higher concentrations of adenine, little further hypoxanthine release was observed, despite less than 20% depletion of intracellular [14C]IMP.

The effect of oxygen on hypoxanthine release induced by adenine was investigated. Deoxygenated red cells were enriched with [14C]IMP as described. A portion of suspension was aerated briefly to reintroduce oxygen, then aliquots of both suspensions were incubated in the presence or absence of 0.2 mM adenine. The ability of excess adenine to induce release of hypoxanthine from IMP-enriched cells (Fig. 8A) was abolished at low oxygen tension (Fig. 8B).

Fig. 8. Effect of oxygen on adenine-induced release of hypoxanthine from IMP-enriched cells. Cells were suspended at pH 7.1 in medium containing 5 mM Pi and 10 μM [14C]hypoxanthine and equilibrated with helium to decrease oxygen tension. After quantitative conversion of [14C]hypoxanthine to [14C]IMP, a portion of suspension was reequilibrated with air, then both aerobic (A) and anaerobic (B) suspensions were incubated in the presence (closed symbols) or absence (open symbols) of 0.2 mM unlabeled adenine. Samples were removed at timed intervals, and the medium [14C]hypoxanthine concentration was measured. The oxygen tension (pO2) of the air (A) and helium (B) equilibrated suspension was 8.5 and 4.3 kPa, respectively.

The effect of xanthine oxidase on adenylate nucleotide catabolism was studied, since ATP is quantitatively the most important red cell purine. The adenylate nucleotide pool of red cells was labeled by incubation with 20 μM [14C]adenine until uptake of label was complete, and corresponded to 40 nmol of [14C]adenylate nucleotide per ml of cells. Analysis of a cell extract showed that the label was virtually confined to ATP and ADP (Fig. 9). The cells were suspended in fresh medium, and accumulation of label in the medium was measured and is shown in Fig. 10. Xanthine oxidase markedly enhanced labeled purine release, which was unaffected by the presence of excess superoxide dismutase (6.4 × 10⁴ units/ml). The extent of [14C]purine accumulation in the medium after 2 h was approximately 4 nmol/ml of cells, equivalent to 10% of the total adenylate nucleotide pool initially present in the cells. [14C]Purine species present in the medium at the end of the incubation were identified by reverse phase HPLC (Fig. 11). In the absence of xanthine oxidase, [14C]hypoxanthine release was small, with most of the radioactivity accounted for.
physiological (8). The enhancement of PRPP synthesis
mined by PRPP synthetase activity, but depends also on the
This inhibition is thought to account for the low levels
PRPP (generally well below 5 nmol/ml of cells) found in
under physiological conditions by ADP and 2,3-BPG (11).
was markedly enhanced and accounted for the bulk of the
rate of PRPP consumption in the hypoxanthine-guanine
by supraphysiological concentrations of inorganic phosphate
is attributed to partial relief of inhibition by Pi (11).
We propose that PRPP accumulation is not solely deter-
the literature for this concept. Inherited deficiency of hypo-
regulation of PRPP synthesis in red cells is currently
believed to operate at the level of PRPP synthetase which
has high intrinsic catalytic activity but is strongly inhibited
under physiological conditions by ADP and 2,3-BPG (11).
This inhibition is thought to account for the low levels of
PRPP (generally well below 5 nmol/ml of cells) found in
circulating erythrocytes and in erythrocytes incubated at
physiological [Pi] (8). The enhancement of PRPP synthesis
by supraphysiological concentrations of inorganic phosphate
is attributed to partial relief of inhibition by Pi, (11).
We propose that PRPP accumulation is not solely deter-
mined by PRPP synthetase activity, but depends also on the
rate of PRPP consumption in the hypoxanthine-guanine
phosphoribosyltransferase reaction. There is some support in
the literature for this concept. Inherited deficiency of hypo-
xanthine-guanine phosphoribosyltransferase, and of purine
nucleotide phosphorylase which provides the hypoxanthine
substrate for hypoxanthine-guanine phosphoribosyltransfer-
ase, is associated with increased erythrocyte PRPP concentra-
tion, while in orotate and adenine phosphoribosyltransfer-
ase deficiency, PRPP levels are normal (8, 19). There is no
observation between erythrocyte PRPP concentration and
PRPP synthetase activity in different mammalian species,
whereas a reciprocal relationship exists between hypoxan-
thine-guanine phosphoribosyltransferase activity and PRPP
concentration (20). In their studies of human erythrocytes
infected with the malaria parasite, Plasmodium falciparum, Roth et
al. (21) noted a sudden, greater than 50-fold increment in
the PRPP content of infected red cells. Since the purine
requirement of the parasite is supported entirely by hypoxan-
thine derived from the host cell (22), we propose that seque-
tration of hypoxanthine by the parasite limits hypoxanthine-
guanine phosphoribosyltransferase activity in the erythrocyte
and permits PRPP to accumulate.
We have demonstrated the ability of xanthine oxidase to
promptly deplete intracellular hypoxanthine, which appears
in the medium as urate. Xanthine oxidase causes PRPP to
accumulate under conditions of alkaline pH and low Pi con-
centration which are not usually associated with such accu-
cumulation (11, 13). Uptake of adenine, guanine, and orotate is
enhanced by xanthine oxidase, apparently due to its stimu-
lation of PRPP levels. We suggest that at alkaline pH or
physiological Pi concentrations, PRPP synthesis is occurring
at a rate comparable to the rate of accumulation of PRPP
seen in the presence of xanthine oxidase, but that synthesis
is matched by PRPP consumption in the hypoxanthine gua-
nine phosphoribosyltransferase reaction. This maintains a
steady state PRPP concentration close to zero. The ability of
IMP to recycle to hypoxanthine by the sequential action of
purine 5'-nucleotidase and nucleoside phosphorylase creates
the potential for a small pool of hypoxanthine to consume a
much larger pool of PRPP. Inhibition of purine-5'-nucleotidase
by Pi, and stimulation by 2,3-BPG could alter the rate of
recycling and thereby influence PRPP levels.
Van der Berge and co-workers (15) have demonstrated the
presence of a purine-specific 5'-nucleotidase from erythro-
cytes, which is maximally stimulated by 2,3-BPG at well below
4 mM, the prevailing concentration of this metabolite in red
cells. However, Bunn et al. (14) have shown that much of this
2,3-BPG is bound to hemoglobin, so that the concentration of
free 2,3-BPG is appreciably lower and varies with the degree
of hemoglobin oxygenation. Thus, purine-5'-nucleotidase in
vivo may well be influenced by prevailing intracellular Pi, and
free 2,3-BPG levels.
Adenine uptake into adenylate nucleotides is enhanced at
low oxygen tension. We propose that since 2,3-BPG binds
preferentially to deoxygenated hemoglobin, the resulting fall
in the free concentration of this metabolite decreases 5'-
nucleotidase activity and retards hypoxanthine regeneration
from IMP. This slows PRPP consumption by hypoxanthine-
guanine phosphoribosyltransferase and makes more PRPP
available for adenine salvage.
We have demonstrated that release of hypoxanthine from
intracellular IMP is dependent on prior depletion of PRPP.
This is observed whether PRPP depletion is achieved by
alkalinizing cells or by adding sufficient exogenous adenine
and indicates that, if PRPP is present, hypoxanthine is prefer-
entially recycled rather than released. The mechanism of the
PRPP depletion induced by alkalinization is interesting.
Dontenw et al. (4) have shown that alkalization enhances
adenylate nucleotide catabolism to hypoxanthine 20-fold, and
that catabolism proceeds via IMP rather than adenosine. We believe that the accelerated rate of hypoxanthine formation is responsible for the steady decline we observe in PRPP levels.

Salerno et al. (23) have proposed that facilitation of IMP conversion to hypoxanthine by adenosine can be explained by reversal of the hypoxanthine-guanine phosphoribosyltransferase reaction, in which IMP and PP, combine to form PRPP and hypoxanthine. This reaction direction is favored by removal of PRPP by adenosine. Such a mechanism should yield a smooth curvilinear relationship between hypoxanthine efflux and adenosine concentration. We find a discontinuous relationship, where a minimum concentration of adenosine is required to initiate hypoxanthine efflux. We suggest that adenosine elicits hypoxanthine release by consuming PRPP in the adenine phosphoribosyltransferase reaction, thereby preventing recycling of hypoxanthine formed from IMP. Once PRPP has been depleted, hypoxanthine release is independent of further increases in adenine concentration and depends entirely on purine-5’-nucleotide activity. Oxygen depletion abolishes hypoxanthine release from IMP in cells exposed to adenine. This is difficult to explain in terms of the proposed direct conversion of IMP to hypoxanthine catalyzed by hypoxanthine-guanine phosphoribosyltransferase (23). We explain the oxygen effect by a decrease in free 2,3-BPG to levels insufficient to sustain purine-5’-nucleotide activity. Activation of purine-5’-nucleotide by 2,3-BPG also accounts for the decreased rate of PRPP accumulation observed in human erythrocytes equilibrated with oxygen, compared to anerobically incubated controls (24).

Xanthine oxidase causes hypoxanthine release from IMP without the necessity for prior PRPP depletion. We propose that it does so by causing hypoxanthine efflux before salvage to IMP can occur. This accounts for the slower decline in intracellular PRPP we observe in the presence of xanthine oxidase.

Adenylate nucleotide catabolism is likewise sensitive to xanthine oxidase. In our experiments, approximately 10% of the adenylate nucleotide pool of cells was degraded to hypoxanthine and appeared in the medium after a 2-h incubation with xanthine oxidase. This was approximately 30-fold greater than the rate of hypoxanthine efflux observed in the absence of the enzyme. Thus, it appears that, if hypoxanthine is promptly removed from the plasma in vivo by, for example, uptake into nucleated cells, red cells may release hypoxanthine at a rate of 70 nmol per ml of cells, or 5% of their total adenylate nucleotide pool, per h.

Xanthine oxidase may enhance purine catabolism by the generation of superoxide radicals. The abundance of superoxide dismutase in red cells, and the failure of added superoxide dismutase to materially alter the results obtained with xanthine oxidase alone, renders this explanation unlikely.

The ability to profoundly deplete intracellular hypoxanthine with xanthine oxidase raises the possibility of a novel therapeutic approach to the treatment of malaria, an infection of human erythrocytes with protozoan parasites which are totally dependent on host hypoxanthine for their purine requirements (22). Bungener showed that allopurinol, an inhibitor of xanthine oxidase, enhanced growth of Plasmodium vinckei in rats (25).

In summary, we propose that an oxypurine substrate cycle, operating in human erythrocytes, regulates PRPP accumulation and determines the ability of red cells to take up purine bases and release hypoxanthine. Unidirectional cycle flux is maintained by the hydrolysis of ATP to AMP, so that sensitive regulation is achieved at the expense of metabolic energy consumption (16). Small changes in pH, P, and oxygen tension modulate cycle flux and thereby regulate purine transport between tissues by red blood cells.

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