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 (P_i), 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 P_i concentrations. Hypoxanthine release from intracellular IMP is strictly dependent on PRPP depletion, induced by either alkalizing 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 P_i inhibition and 2,3-bisphosphoglycerate activation of pyrimidine 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 between tissues by red cells (2). Henderson and Le Page (3) demonstrated that, in rats and mice, erythrocytes at high specific activity (1). Since mammalian red cells synthesize PRPP maximally at physiological [P_i] (~1 mM), the low levels of PRPP (l-5 mM) found in normal red cells, or cells incubated at physiological [P_i] (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 P_i (11). The concentration of ribose 5-phosphate in red cells is well below the K_m for PRPP synthetase, estimated at 3 × 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 [P_i]. P_i 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 P_i. Whelan and Bagnara (5) showed that, while IMP dephosphorylation was markedly enhanced by oxygen and inhibited by P_i, ATP catabolism was unaffected by such manipulations.

We have previously described the effects of pH, P_i, and oxygen on red cell hypoxanthine uptake and release (13). Enhanced uptake was observed with high [P_i], low pH, and low P_iO_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 Newsholme (16). An example is the two-component cycle, comprising the glycolytic intermediates, fructose-1,6-bisphosphate and 3-PG, which catalyze the conversion of 3-PG to fructose-1,6-bisphosphate and 3-PG to P_i. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: PRPP, 5-phosphoribosyl 1-pyrophosphate; 2,3-BPG, 2,3-bisphosphoglycerate; HPLC, high pressure liquid chromatography; MES, 4-morpholineethanesulfonic acid.
6-phosphate and fructose 1,6-bisphosphate. This cycle permits sensitive control of glucose uptake and release in liver tissue by allosteric effects of phosphofructokinase activity, including ATP, AMP, P1, 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 activation and the synthesis of purines and pyrimidines in human erythrocytes. A diagram of the oxypurine cycle and its metabolic inter-relationships is presented in Fig. 1.

**EXPERIMENTAL PROCEDURES**

**Materials**—[^14C]Hypoxanthine, [^8-i4C]adenine, [^8-i4C]guanine, [[^6-i4C]IMP], and [[^8-i4C]GMP] (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 C. Merck, Darmstadt, F.R.G; PRPP, superoxide dismutase, and Ficoll-Histopaque from Sigma; and DEAE-SpectraGel 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 leucocytes 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. Concentration of the medium was varied by addition of NaH2PO4 to make the medium 50 mM. To obtain quantitative conversion of[^14C]hypoxanthine and[^14C]adenine to labeled IMP and adenylic nucleotides, respectively, cells were incubated at pH 7.0 in 5-10 mM P1 with the appropriate[^14C]purine base, either stimulatory (+) or inhibitory (−).

**Quantitation of Oxypurine Cycling**—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. (21). The supernatant medium was deproteinized with 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 assayed for radioactivity, 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 K2CO3, and 20 μl of supernatant was analyzed by HPLC.

**Manipulation of Erythrocyte Oxygen Tension**—Oxygen tension (PO2) was decreased or increased by bubbling helium 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 bottles to prevent changes in PO2. PO2 measurements were performed on a Radiometer ABL blood gas analyzer.

**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 centriufugation at 10,000 × g for 30 s, and 50 μl of supernatant was mixed with 15 μl of 2.5 M KClO4, and 40% (v/v) of a 0.6 M perchloric acid was added. The denatured protein was removed by centrifugation, and 50 μl of supernatant was neutralized with 5 μl of 2.5 M K2CO3. The KCIO4 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 69%.

**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 37°C with 50 mM Mops, pH 6.5, 30 μM MgCl2, 200 μM[^14C]hypoxanthine, 57 Ci/mol, and 40% (v/v) of a 0.6 M perchloric acid. The extract was neutralized with K2CO3, and its isoinine 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 model SP 8800 solvent delivery system. 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 HCOOH, adjusted to pH 4.5 with H3PO4, to 1 M NaH2PO4, adjusted to pH 2.7 with 10000. At a flow rate of 2 ml/min, the high concentration eluant was maintained for 6 min to allow elution of ATP and GTP. Reverse phase chromatography was performed on a Vydac 201 HS 54 (C-18) column. The polar eluant was 1 M KH2PO4 in water (solvent A), and the nonpolar eluant acetone/tritritile, 25% (v/v), in water (solvent B). A linear gradient was developed over 10 min from 0–100% solvent B at a flow rate of 1.0 ml/min. The reaction mixture were removed at hourly intervals and deproteinized with 0.6 M perchloric acid. The extract was neutralized with K2CO3, and its inosine content was quantitated by reverse phase HPLC, using external inosine standards.
with buffer A for 5 min, then a linear gradient was developed to 100% B over 3 min. 100% B was maintained until all purine bases had eluted. This system permitted nucleotide separation from nucleosides and bases, as well as separation of adenylate and bases from one another. Ultrafiltration and nonpolynucleotide eluates were filtered on MilliPore membrane filters HATF 047 EP and HVLP 047 00, respectively, and degassed with helium prior to use. The absorbance of the column effluent was monitored at 250 nm, using a Spectra Physics SP 545 UV/vis absorbance detector and elution positions of purine species identified with external standards.

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 [14C]hypoxanthine, then xanthine oxidase was added. Changes in intracellular and extracellular [14C]purine concentrations were monitored. Xanthine oxidase caused immediate depletion of both medium and cellular [14C]hypoxanthine, to levels below 0.1 μM. In the medium, more [14C]urate accumulated than [14C]hypoxanthine, whereas in the cells, [14C]hypoxanthine depletion was not immediately accompanied by a concomitant increase in [14C]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 Pi concentration. Inclusion of xanthine oxidase in the medium abolished this dependence on pH and Pi; PRPP accumulated irrespective of pH and in the absence of added Pi. This suggests that in intact cells Pi may be required, not only to activate PRPP synthesis, but also to inhibit PRPP consumption by hypoxanthine oxidase.

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 14C-labeled adenine, guanine, orotate, and adenosine into nucleotides is shown in Fig. 3. Erythrocytes were incubated in medium containing 50 μM 14C-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 [14C]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 adenylate nucleotides in cells incubated anaerobically (Fig. 4B), when compared to the aerobically incubated control (Fig. 4C).
under “Experimental Procedures,” then resuspended in fresh medium devoid of Pi. At pH 6.94, negligible [3H]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 [3H]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 [3H]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 [3H]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 PRPP 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 [3H]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 [3H]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. 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 [W]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 [W]hypoxanthine concentration (open symbols) and total intracellular [W]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 [W]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 [W]hypoxanthine occurred at medium adenine concentrations lower than 20 μM. Between 20 and 40 μM adenine, [W]hypoxanthine efflux increased rapidly, while at higher concentrations of adenine, little further hypoxanthine release was observed, despite less than 20% depletion of intracellular [W]IMP.

The effect of oxygen on hypoxanthine release induced by adenine was investigated. Deoxygenated red cells were enriched with [W]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 [W]hypoxanthine and equilibrated with helium to decrease oxygen tension. After quantitative conversion of [W]hypoxanthine to [W]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 [W]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 [W]adenine until uptake of label was complete, and corresponded to 40 nmol of [W]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 [W]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. [W]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, [W]hypoxanthine release was small, with most of the radioactivity accounted
for by [14C]ATP and [14C]ADP. In the presence of xanthine oxidase, [14C]hypoxanthine release, measured as [14C]urate, was markedly enhanced and accounted for the bulk of the medium radioactivity. Inclusion of superoxide dismutase made little difference to the pattern of labeled purine release.

**DISCUSSION**

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 [P_i] (8). The enhancement of PRPP synthesis by supraphysiological concentrations of inorganic phosphate is attributed to partial relief of inhibition by P_i (11).

We propose that PRPP accumulation is not solely determined 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 hypoxanthine-guanine phosphoribosyltransferase, and of purine nucleotide phosphorylase which provides the hypoxanthine substrate for hypoxanthine-guanine phosphoribosyltransferase, is associated with increased erythrocyte PRPP concentration, while in orotate and adenine phosphoribosyltransferase deficiency, PRPP levels are normal (8, 19). There is no correlation between erythrocyte PRPP concentration and PRPP synthetase activity in different mammalian species, whereas a reciprocal relationship exists between hypoxanthine-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 decrement in the PRPP content of infected red cells. Since the purine requirement of the parasite is supported entirely by hypoxanthine derived from the host cell (22), we propose that sequestration 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 P_i concentration which are not usually associated with such accumulation (11, 13). Uptake of adenine, guanine, and orotate is enhanced by xanthine oxidase, apparently due to its stimulation of PRPP levels. We suggest that at alkaline pH or physiological P_i 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-guanine 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 P_i 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 erythrocytes, 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 P_i 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 observed whether PRPP depletion is achieved by alkalinizing cells or by adding sufficient exogenous adenine and indicates that, if PRPP is present, hypoxanthine is preferentially recycled rather than released. The mechanism of the PRPP depletion induced by alkalinization is interesting.

Bontems et al. (4) have shown that alkalinization 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 adenine 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 adenine. Such a mechanism should yield a smooth curvilinear relationship between hypoxanthine efflux and adenine concentration. We find a discontinuous relationship, where a minimum concentration of adenine is required to initiate hypoxanthine efflux. We suggest that adenine elicits hypoxanthine release by consuming PRPP in the adenine phosphoribosyltransferase reaction, which is approximately 30-fold more efficient than the xanthine oxidase alone, rendering this explanation unlikely.

The ability to profoundly deplete intracellular hypoxanthine by adenine 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'-nucleotidase by 2,3-BPG also accounts for the decreased rate of PRPP accumulation observed in human erythrocytes equilibrated with oxygen, compared to anaerobically 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 infestation 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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