Review

Disorders of Purine Metabolism in Human Erythrocytes in the State of Lead Contamination

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Abstract

Although many countries now have programs to lower the levels of lead in the environment, human exposure to Pb remains of concern to public health officials worldwide. The mechanisms of lead toxicity are still not fully understood, but recent findings have recognized the significance of the lead-induced impairment of the cell energy metabolism. This review outlines recent hypotheses and evidence on the role of nucleotide purines in erythrocyte metabolism regulation. It also describes the resynthesis and decomposition of purine nucleotides in erythrocytes, lead distribution in blood and its influence on purine conversion pathways and both glycolytic and pentosophosphate pathway enzymes in erythrocytes.

Keywords: Energy metabolism, erythrocytes, lead ions, purine nucleotides, purine conversion pathway

Universal Meaning of Purine Metabolism

Purine nucleotides are widespread compounds in the organic world and take part in all metabolic processes in cells [1, 2, 3, 4]:

1. ATP is an energy carrier in cells. It connects the processes where the energy is produced with those where the energy is consumed, including active transport, contraction of muscles and maintaining the ion gradient.
2. They are the RNA and DNA precursors and are substrates in reactions catalyzed by RNA and DNA polymerase.
3. They are the substrates for the biosynthesis of the signal molecules second messengers such as: cyclic nucleotides – cAMP, cGMP and diadenosine polyphosphates (Ap_nA).
4. They are precursors of coenzymes: NAD^+, $NADP^+$, FAD and CoA
5. Derivatives of nucleotides are non-direct compounds in many biosyntheses, for example S- adenosyl methionine (SAM).

6. They are allosteric effectors – many metabolic pathways are controlled by the intracellular ATP concentration, e.g. glycolysis; GTP takes part in moving macromolecules, such as the ribosome-based peptide chains; ADP-ribose takes part in post-translate modifications of certain proteins (Fig. 1).

Role of the Nucleotide Purines in Erythrocyte Metabolism Regulation

The most important indicator of the energy status of cells is the adenylate (guanylate) energy charge – AEC (GEC) [5]. Adenylate energy charge (AEC), expressed by the ratio of the high to low energy nucleoside phosphates, is another parameter describing the cell energy status.

The values of AEC (GEC) range from 0, when the total nucleotide pool is AMP (GMP), to 1, when all the adenylate (guanylate) nucleotides are present in the form of ATP (GTP).

In the organism's homeostasis AEC (GEC) is strictly controlled and their value ranges from 0.8 to 0.9. AEC (GEC) value close to 1 suggests the high potential of metabolic energy. The values 0.55-0.75 indicate the negative in-
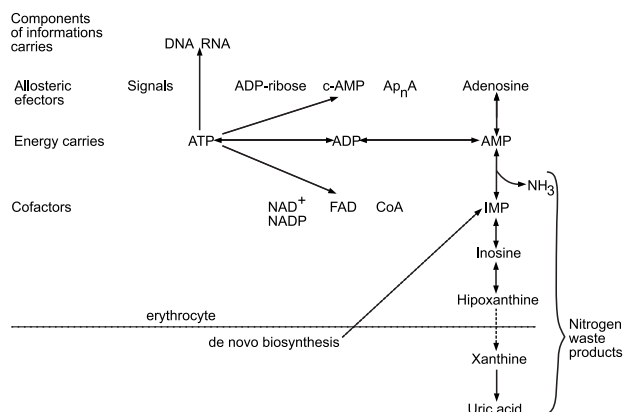
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Fig.1 Scheme of some adenylate containing molecules and their biological significance [4].

fluence of the stress factor inducing energy expenditure in the direction of the defensive mechanisms, and the values below 0.5 accompany the death of cells [6, 7, 8, 9, 10].

In the regulation of the erythrocytes metabolism the most important compounds are ATP, ADP and AMP. The energy released in the process of enzymatic hydrolysis of the ester bond with ATP is consumed in glucose phosphorylation on the first stage of glycolysis, in the membrane and cytoplasmatic proteins phosphorylation, membrane lipids acylation, in keeping iron ions in the reduced state, gluthatione and pyridine nucleotides syntheses and in the active transport of ions.

There are three energy transporting systems responsible for the regulation of energy metabolism: adenosine phosphates (ATP, ADP and AMP), $NADP^+$ and NAD^+ [11]. They undergo reversible transformations in red blood cells, due to the processes of glycolysis and the penthosophosphate pathway [12].

The penthosophosphate pathway provides NADPH, the reduced pyridine coenzyme, responsible for the reductive-oxidative balance, and also 5-phosphoribosyl-1-pyrophosphate, necessary for reconstructing ATP in erythrocytes. The reduced coenzyme NADH is produced in the oxidation of 3-phosphoglycerate aldehyde in the main pathway of the glucose conversion [13].

Purine Nucleotides Metabolism
Resynthesis of Purine Nucleotides in Erythrocytes

Purine nucleotides may be synthesized in cells de novo or reconstructed from already existing free purine bases through the salvage reactions (reutilization). Mature erythrocytes cannot synthesize 5-phosphoribosylamine and that is why the synthesis of nucleotides de novo is not possible in these cells [14].

Adenine nucleotides are 70-80% of all free erythrocyte nucleotides [11], and their precursors in red blood cells are adenine and adenosine, the transport of which through the erythrocyte membranes takes place through the facilitated diffusion [14, 15].

The synthesis of adenine nucleotides in erythrocytes may be carried by:
1. Pathway dependent on 5-phosphoribosyl-1- pyrophosphate (PRPP), requiring the presence of adenine phosphoribosyltransferase (APRT) [16]
2. Adenosine pathway independent from PRPP, requiring the presence of adenosine kinase (AdoK) [17]
3. S-adenosylhomocysteine hydrolase (SAH) pathway [18]

The Michaelis constants for adenine phosphoribosyltransferase are 1-5 μM in case of adenine and 2 μM for adenosine kinase in case of adenosine, which indicates the greater importance of PRPP-dependent adenylate pathway in comparison with the adenosine one [17]. The synthesis of PRPP in erythrocyte is presented in Fig. 2.

Adenine Phosphoribosyltransferase (APRT)

In the result of PS (synthase of 5-phosphoribosyl-1-pyrophosphate EC 2.7.6.1) activity on ribose-5-phosphate (Rib-5-P) with the participation of ATP, the PRPP is produced (5-phosphoribosyl-1-pyrophosphate). In the following reaction APRT (EC 2.4.2.7 adenine phosphoribosyltranspherase) catalyzes the fixing of phosphoribosyl rest by adenine. The resultant inorganic pyrophosphate (PPi) is decomposed into phosphate (Pi) by the inorganic pyrophosphatase, which moves the balance of the adenine phosphoribosylation into right and makes it irreversible. The APRP activators are divalent cations, the most effective of which is Mg^{2+} with its concentration twice higher than PRPP [16].

The reaction is feedback-inhibited by the product – AMP. The decisive factor determining the speed of the reaction at certain adenine concentration is the ratio of concentrations [PRPP]/ [ATP]. The competitive inhibitors are ADP and ATP that connect with adenine and AMP at certain places. GMP is a strong allosteric inhibitor, although due to its low intracellular concentration it is not certain if it plays a regulating role *in vivo* [16].

Adenosine Kinase (AdoK)

Under the influence of adenylate kinase AK (EC 2.7.4.3), the moiety of AMP reacts with the molecule of
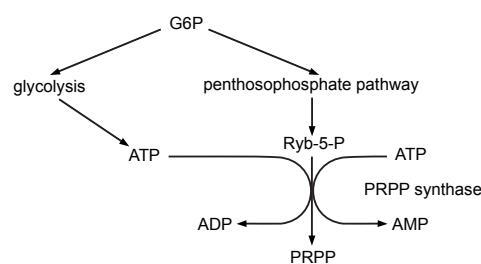

Fig. 2. The synthesis PRPP in erythrocytes.

ATP. This gives ADP, which in turn undergoes phosphorylation into ATP in the process of glycolysis [17].

PRPP serves also as a substrate in reaction with hypoxanthine or guanine catalyzed by HGPRT (EC 2.4.2.8 hypoxanthine-guanine phosphoribosyltransferase), which results in the production of IMP or GMP [17]. On the pathway of GMP synthesis, IMP undergoes oxidation, with the participation of dehydrogenase IMP (EC 1.1.1.205) into XMP (xanthosine-5'-monophosphate). Under the influence of GMP synthase activity (EC 6.3.5.2) XMP binds the amino group from glutamine, which produces GMP [19, 20].

The synthesis of purine nucleotides is regulated by the feedback inhibition. The PRPP (5-phosphoribosyl-1-pyrophosphate synthase) synthesizing enzyme undergoes partial inhibition through high concentration of purine nucleotides, which act as allosteric regulators. PRPP also takes part in the pyrimidine nucleotides and NAD^+ synthesis, so it undergoes the cumulated feedback inhibition by the compounds produced on various pathways of syntheses with the participation of PRPP. AMP and GMP, the final products of those processes, act synergically in the enzyme inhibition [17].

Two mechanisms regulate conversion of IMP to GMP and AMP. AMP inhibits the conversion of IMP into adenylosuccinate and its direct precursor [17]. In a similar way GMP is the inhibitor of IMP conversion into the direct precursor – XMP. GTP is a substrate in the synthesis of AMP and AMP is a substrate in GMP synthesis. That mutual substrate dependence serves to maintain the balance of adenine and guanine ribonucleotides synthesis [17].

S-adenosylhomocysteine Hydrolase (SAH)

Smolenski et al. [21] suggest that another ATP resynthesis mechanism is possible in erythrocytes. It happens through the S-adenosylhomocysteine hydrolase SAH (EC 3.3.1.1). This enzyme catalyzes reversible S-adenosylhomocysteine hydrolysis into adenosine (Ado) and L-homocysteine. In the reaction an unstable indirect metabolite is produced (a 3-ketonucleoside) whose glycosilic bond may undergo an intrinsic, non-enzymatic hydrolysis. The conversion takes place in an especially intense fashion when the substrate is deoxyadenosine (dAdo), and the products are deoxyribose (dRib) and adenine, which in reaction with APRT may be transformed into ATP [21].

S-adenosylmetionine may also undergo decarboxylation in the polyamine pathway. The product of that reaction serves as an aminopropane group donor in synthesis of spermidine and spermine. 5'-deoxy-5'-methyltioadenosine (5'–MT–Ado, MTAR) is produced, which is a substrate for 5'-MT-Ado phosphorylase (EC 2.4.2.28); the products of the reaction catalyzed by that enzyme are adenine and 5'-deoxy-5'-methyltioribose-1- phosphate (5'MT-Rib-1-P) [16, 21].

On the other hand, adenine could be released from certain adenosine analogs through methyltioadenosine 5' MT–Ado (EC 2.4.2.28) phosphorylase. That enzyme is also present in erythrocytes but the reaction may take part only at high Ado concentration in the ischemia of tissues [21].

The above authors have shown that ATP cannot be resynthesized from hypoxanthine because a mature erythrocyte does not have enzymes transforming IMP into AMP [21].
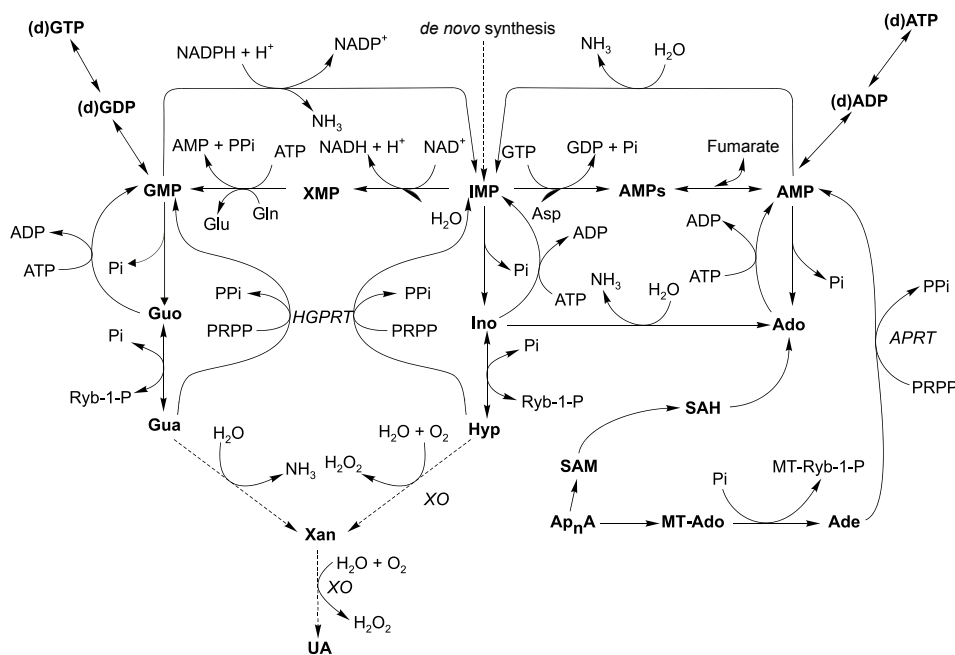

Fig. 3 Pathway of purine metabolism in erythrocytes [16].

Decomposition of Purine Nucleotides in Erythrocytes

In erythrocytes, catabolism of adenine nucleotides depends on the balance of a reaction catalyzed by adenylate kinase AK (EC 2.7.4.3) that regulates the proportions between ATP, ADP and AMP (Fig.3). The nucleoside bisphosphokinase (EC 2.7.4.6) and guanylate kinase (EC 2.7.4.8) regulate the balance between pools of guanine nucleotides. The aim of these reactions is to maintain the charge of adenylates (guanylates) in a cell [22, 23].

5'nucleotidase

Decomposition of AMP into hypoxanthine in human erythrocytes may take place in two ways [4, 22, 23, 24, 25, 26]. Catabolism going through the first path is based on the AMP dephosphorylation into adenosine in the reaction catalyzed by the 5'nucleotidase (EC 3.1.3.5), and then adenosine deaminase into inosine (Ino) in the reaction catalyzed by adenosine deaminase (EC 3.5.4.4). Ado may undergo rephosphorylation into AMP, with the participation of ATP in the reaction catalyzed by adenosine kinase (Ado K) (EC.2.7.1.20) [22, 24, 25, 26, 27, 29].

AMP Deaminase

The second path is started by the reaction of AMP deamination into IMP catalyzed by the AMP deaminase (EC 3.5.4.6) and then in the next reaction IMP undergoes dephosphorylation into Ino under the influence of 5'nucleotidase [22, 26, 30, 31]. Ino under the influence of purine nucleoside phosphorylase (and in the presence of Pi) transforms into Hyp together with the simultaneous production of ribose-1-phosphate (Rib-1-P). That compound reacts with phosphoribomutase, which gives ribose-5-phosphate (Ryb-5-P), which provides metabolites for glycolysis in transformations of penthosophosphate cycle or, under the influence of PRPP synthase and the participation of ATP, results in PRPP [25, 29]. AMP catabolism in the physiological conditions proceeds mainly through deamination (the second path) [27, 32].

It has been shown that AMP deaminase has the characteristics of an allosteric protein [33, 34]. It is suggested that there exist both active and inactive enzyme forms in vivo; however, the activation takes place under the influence of a substrate. It has been stated that the reaction's products (IMP and NH_3) inhibit the reaction; inhibition through IMP having the competitive character in relation to AMP, and inhibition through ammonia has a mixed character – competitive and non-competitive [33]. The deaminase allosteric effectors are Na^+ and K^+ ions which at the concentration of 100–150 mM, similar to the physiological concentration in cells, stabilize the AMP deaminase molecule in a conformation in which it is able to bind with the physiological ligands: AMP, ATP, GTP, Pi [35]. GTP inhibits the enzyme feedback, as a product of a transformation cycle, the part of which is AMP deaminase [35]. ATP accelerates AMP deamination, pre-

venting the AEC value from decreasing, through shifting the balance in the direction of ATP production, which in turn accelerates the rate of anabolic transformations. The consumption of ATP in anabolic conversions leads to the release of a considerable amount of inorganic phosphate that inhibits AMP deaminase and decreases the absolute amount of ATP in a cell [33].

However, it is AEC and not the absolute values of particular nucleotide concentrations in a cell that is the decisive factor in the regulation of the AMP deaminase [35]. The reaction catalyzed by the AMP deaminase is inhibited also by the compounds blocking the sulfhydryl (SH) groups; among others lead ions [35, 36, 37, 38, 39].

The AMP catabolism pathway into adenosine, catalyzed by the 5'-nucleotidase is equally important, regarding the role of adenosine in the organism and its wide influence on the functions and metabolism of most tissues [4, 23, 25].

There are two basic forms of that enzyme: cN–I and cN–II. In erythrocytes 5'–nucleotidase cN–II, stimulated by the 2,3-BPG prefers the IMP dephosphorylation, and the cN–I reveals greater activity in relation to AMP. The simultaneous action of ADP and 2,3-BPG stimulates the enzyme in a four times greater way than the each of the compounds alone [40]. Also, the low glucose concentration may stimulate the AMP dephosphorylation (in that case 75% of AMP is dephosphorylated through that pathway and only 25% is deaminized) [27, 31, 41, 42, 43].

The adenine nucleotide metabolism in erythrocytes is connected strictly with glycolysis and the penthosophosphate cycle. Therefore, factors which inhibit these processes are also responsible for disorders in the metabolism of the nucleotides [44, 45]. At the same time some of the glycolysis metabolites, among others glucose-6-phosphate, fructose 1,6-biphosphate, 2–phospho–glycerin acid, 2, 3–bisphosphoglycerate and phosphoenol pyruvate inhibit the AMP deaminase activity taken from various animal tissues [46].

GMP in erythrocytes may undergo dephosphorylation with the participation of 5'nucleotidase into Guo. The purine nucleoside phosphorylase (EC 2.4.2.1) catalyzes further Guo phosphorolysis reaction into guanine – Gua with the simultaneous release of Rib-1-P. Due to the fact that erythrocytes do not have the guanase enzyme, guanine is the final product of the guanine nucleotides metabolism in erythrocytes. Guanine may be included in the conversion cycle as a substrate for HGPRT [43, 48, 49].

Lead Ion Influenced Disorders in the Erythrocyte Energy Metabolism

Lead Distribution in Blood

Lead present in blood is mainly located in erythrocytes, since only 1% of Pb remains in serum [50]. 90% of lead in erythrocytes is present in the erythrocyte cytoplasm and 10% is connected to the membrane, mainly lipids and lipoproteins [51, 52]. The distribution of Pb

into plasma and erythrocytes is due to the binding of lead with the cytoplasm elements; transport through the erythrocyte membrane [51, 52] and excretion through the calcium pump [52]. The toxic influence of lead upon erythrocytes comes from its ability to create complexes with the negatively charged ligands, most notably the sulphydryl, carboxyl and imidasole enzyme groups and other proteins [53, 54].

Mechanisms of the Toxic Effect of Lead

The influence of Pb may be so strong that it results in changes in the proteins' conformation, through the hydrogen bind cleavage or moving cations from their activity sites [55, 56]. In the case of free sulfhydryl groups of amino acids belonging to the active enzyme center, lead may block the thiol groups and in that way make it impossible for S-S bridges to be formed. Those bridges are necessary for the catalytic center to function and the lack of them may affect the catalytic capability of the enzyme and eventually lead to its deactivation [57, 58].

In the case of metalloenzymes containing zinc or magnesium in their prosthetic groups, it is the competitive binding of lead in the place of those metals [59, 60, 61] that results in the disorders in these enzymes' activity and cell metabolism. [53, 62, 63, 64].

Another mechanism is the ability of Pb^{2+} to use the same pathways of entry as Ca^{2+}. It has a double detrimental effect: first $Pb2^+$ interferes with and damages Ca^{2+} transport systems; secondly, Pb^{2+} takes advantage of these systems to penetrate into the cytoplasm and carry on its destructive mimicking action by occupying Ca^{2+} binding sites on numerous Ca^{2+}-dependent proteins that reside intracellulary [65].

Lead Influence on Purine Conversions
Pathways in Erythrocytes

The toxic influence of lead on humans and animals has been examined for many years [51, 66, 67, 68], although not too many reports have concerned the lead-induced changes in cell energy metabolism [7, 50, 55, 63, 70, 71, 72]. The few reports on the energy metabolism concerned lead-induced impairments of enzyme activity of glycolysis and pentosophosphate pathways [70, 72, 73]. Purine conversion pathways in erythrocytes (Fig. 3) are in a close relation with glycolysis and pentosophosphate pathway since they are the source of highly energetic compounds and intermediates of the purine conversions in erythrocytes. Therefore in the following parts we will present the effect of lead on the glycolysis and pentosophosphate pathways enzymes activity.

Glycolytic regulating enzymes: hexokinase (EC 2.7.1.1), phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) directly influence the nucleotide concentration, essential for performing the basic physiological functions of the erythrocytes. Even small lead-induced changes in these enzymes' allosteric modifiers may lead

to changes in enzymatic reaction rate and substantially affect the course of glycolysis, which may affect the ATP, ADP, AMP and 2, 3-BPG concentration in erythrocytes [12, 13]. Glyceraldehyde-3-phosphate dehydrogenase and pyruvate dehydrogenase, two other glycolytic enzymes, are also sensitive to lead.

Lead Influence on Glycolytic Enzymes

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)

Grabowska and Guminska [63] showed that Pb inhibits glycolysis in red blood cells through inhibiting the glyceraldehyde-3-phosphate dehydrogenase activity, the NAD^+ dependent enzyme. This enzyme contains –SH groups in its catalytic center and Pb may affect its catalytic activity through the aforementioned mechanism. The decreased lactate synthesis in vitro, observed by the authors, was neutralized by the stimulation of Mg^{2+}. This may suggest the additional lead-induced inhibition of the Mg-dependent glycolytic enzymes [63]. The degree of lead-induced ATP concentration decrease in erythrocytes may be the consequence of inhibiting the glycolysis (the only source of ATP in red blood cells), but to the same extent it seems to depend on ATP utilization in normal energy-consuming processes [57, 70]. Grabowska and Guminska [63] suggest that the Pb influence on the ATP concentration is lower than on the lactate synthesis because of the observed inhibition of the ATP consumption by the $Na^+ K^+$ ATPase.

Hexokinase (HK), Pyruvate Dehydrogenase (PDHc), Phosphofructokinase (PFK)

Hexokinase (HK) lead-induced decrease in glycolytic enzymes' activity, among others hexokinase, was observed in studies on the energy metabolism in tissues [56, 74, 75, 76].

In the studies on the activity of pyruvate dehydrogenase (PDHc) and hexokinase (HK) in rat's brain homogenates during incubation in lead acetate (concentration below 5μM) Yun et al. [72] observed the complete inhibition of the examined enzymes. They also observed the inhibiting influence of the micromolar lead concentration on phosphofructokinase (PFK) activity [73], one of the regulating enzymes that controls glucose conversion [77, 78].

Rats chronically exposed to lead were reported to have brains sensitive to micromolar Pb concentrations, on the basis of ratios of lead content in the brain and blood. Those low concentrations inhibited the HK and PDH activity [79]. It is not clear if these low lead concentrations inhibit these enzymes in the physiological conditions, but the rat's brains subjected to lead were observed to have the significant lypoyl dehydrogenase, which is a part of the PDHc complex [76].

Decreased concentration of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) was observed in the male human blood with the Pb-B concentration

5,0–9,8 μM [56]. It suggests that if the glucose and ATP concentrations decrease in the physiological condition, it may lead to the disorders in cell metabolism due to inhibition of HK [56].

Decrease in AEC, TAN and ATP in rat erythrocytes observed even at the lead concentration in blood below 10 μg/dl [8], confirmed by the studies on isolated erythrocytes in vitro [9], indicates that the same changes in the energy metabolism may occur in humans exposed to low, environmental doses of lead [66, 67, 68].

Pyruvate Kinase (PK)

Pyruvate kinase (PK) – the third allosteric enzyme of glycolysis, shows cooperativeness in relation to a substrate (phosphoenolopyruvate PEP), but not to ADP. The enzyme directly controls the glucose utilization pathway, and indirectly the level of adenine nucleotides, 2, 3-BPG and NAD$^+$. Pb inhibits the activity of pyruvate kinase (PK) in erythrocytes; this leads to a decrease in ATP concentration [55]. However, the decrease in ATP is not accompanied by an increase in ADP and AMP in red blood cells [80, 81]. TAN and the total of oxidized and reduced nucleotides (NAD$^+$ + NADH) is also lower in erythrocytes with decreased PK activity [80, 81].

The studies by other authors [55, 70, 81] showed that in erythrocytes with decreased PK activity smaller amounts of ATP, PRPP and NAD$^+$ is produced, which results in a decrease in the total pool of adenine nucleotides [19, 80]. Moreover, the marked ATP concentrations in erythrocytes with the deficiency of pyruvate kinase do not extend the Michaelis constant (0, 4 mM) for adenosine kinase, which impairs salvage path in red blood cells [17].

Decrease in NAD$^+$, which is a co-substrate for glyceraldehydes -3-phosphate dehydrogenase results in the slow down of the reaction catalyzed by that enzyme. The disorders of nucleotide concentrations also affect the correct functioning of these enzymes and, in consequence, glycolysis [71, 80].

Decrease in pyruvate kinase activity also results in an increase in content of 2, 3-BPG in erythrocytes [17, 71]. At high concentrations it is an inhibitor of hexokinase and glucose-6-phosphate dehydrogenase G6PD (EC 1.1.1.49) (also directly inhibited by lead) [71]. Blocking these enzymes inhibits the pentosophosphate pathway and in effect decreases the amount of the produced PRPP. That, in turn, lowers ATP concentration in erythrocytes [19, 70].

Pentosophosphate Pathway Enzymes

Two enzymes of the pentosophsphate pathway: transaldolase (TA) and transketolase (TK), taking part in the synthesis of Rib-5-P are also inhibited by the high concentration of 2, 3-BPG [17]. Since Rib-5-P is a precursor of PRPP, it is an additional mechanism limiting the amount of the produced PRPP in red blood cells exposed to lead. The effect overlapping the aforementioned phenomena is the inhibiting influence of lead on PRPP synthase [17, 71].

Studies of Lachant et al. [73] confirm the inhibiting influence of lead ions both on glycolytic enzyme activity and the pentosophosphate pathway. They observed decrease in the activity of G6PD, 6-phosphogluconate dehydrogenase (6PGD), phosphofructokinase and hexokinase marked in the freshly hemolized erythrocytes, after earlier incubation with lead ions. In these studies, PK appeared to be insensitive to Pb below the level of 200 μM. Lachant [73] suggest that lead acts as non- competitive G6PD inhibitor in relation to G6P.

5'pyrimidine Nucleotidase

Pb was observed to inhibit also 5` pyrimidine nucleotidase (EC 6.3.1.5) activity in people occupationally exposed to lead, experimental animals and erythrocytes in vitro. The inhibition of the enzyme results first in the accumulation of pyrimidine nucleotides, which is associated with the induction of base-absorbent basophilic stippling in erythrocytes [27, 57, 73].

Increased pyrimidine concentrations also inhibit G6PD through competitive inhibition in relation to G6P and non-competitive in relation to NADP [73]. It was also observed that high pyrimidine concentrations in vitro inhibit glycolytic enzymes, which result in NADPH deficiency in erythrocyte and showing pseudo-deficiency of G6PD [57]. Increase of nucleotide phosphate concentration results in the decrease of intracellular pH, which in effect lowers G6PD activity even more and enhances oxidative erythrocyte sensitivity [73].

5'nucleotidase cN-I

Cytozol 5'nucleotidase is present in two isoforms, which are differentiated on the basis of the substrate preference in relation to IMP and AMP, respectively [82, 83]. IMP, specific cytozol 5'nucleotidase, is responsible for inosine release from IMP and requires Mg^{2+} for its activity. ATP and ADP, contrary to ecto 5'nucleotidase, activate this enzyme, and inorganic phosphates are its inhibitor [82]. It was also observed that 5'nucleotidase might be activated by 2, 3-BPG, which prefers IMP phosphorylation, and not AMP [31].

Lead-induced 5'pyrimidine nucleotidase inhibition [31] and observed in our own studies AMP concentration increase [8], with the simultaneous Ado decrease and suggested by other authors [17] impairment of the salvage pathway may give us right to put forward a hypothesis on the inhibition of 5'nucleotidase cN-I (specific AMP) activity. IMP concentration decrease and Ino concentration increase, observed only at very high Pb concentration [8], may also suggest that lead does not influence so strongly the activity of 5'nucleootidase cN-II, (specific IMP).

NAD⁺ Synthase

In our studies [9], we observed a very strong negative correlation between the lead concentration in blood and the decrease of NAD⁺ and NADP in erythrocytes. Zereza et al. [81] confirm the low NAD⁺ concentration in humans with an increase amount of lead in blood, also in vitro. The author stated that it is the lead-induced inhibition of NAD⁺ synthase (EC 6.3.5.1) activity that is responsible for the NAD⁺ decrease in red blood cells.

The enzymes catalyze the transfer of the amino group from glutamine onto the rest of deamido-NAD nicotinian (NAAD) in the presence of ATP, Mg^{2+}, K^+. The resultant NAD⁺ may get phosphorylized into NADP. Pb concentration, inhibiting the enzyme in 50%, was 1, 3 μM Pb. Intact red blood cells incubated in vitro in 50 μM lead acetate showed also complete inhibition of NAD⁺ synthase, which may indicate that inactivation does not take place through the sulphydryl groups [81]. In the same conditions G6PD was insensitive to lead influence, and the activity of 5'pyrimidine nucleotidase decreased by 30% [81]. In patients with the increased Pb-B level (34-72 μg/dL) Zerez and Tanaka [81] observed decrease in NAD⁺ synthase activity compared to patients with Pb-B below 10 μg/dL [81]. In subacute lead contamination in rats, erythrocytes displayed lower level of tryptophan. Tryptophan, transformed into nicotinic acid, is used for the synthesis of NAD⁺ and NADP. Zerez, Tanaka [81] suggest on the basis of the aforementioned data that NAD⁺ synthase is an enzyme very sensitive to lead and may be an indicator of lead exposition even at very low levels of Pb.

Oxidative-Reduction Enzymes

Many authors have observed that Pb affects the activity of enzymes responsible for maintenance of oxidative-reduction balance in cells: methemoglobine reductase, glutate reductase, G6PD, catalase, LDH, SOD. However, reports on their sensitivity to lead have been vary [58, 84, 85, 86].

Erythrocytes of workers occupationally exposed to Pb were reported to have the activity of glutate peroxidase and SOD increased by 3.3 and 2.2 times, respectively, than in workers not exposed to Pb. Protoporfirine IX and methemoglobine concentrations were two times higher. The authors interpret the increase in the activity of antioxidative enzymes as the defense reply of the organism against the increase of the synthesis of oxygen active forms [87, 88, 89].

It was also observed that cells subjected to oxidative stress have lower ATP concentration [87]. Adenosine, in turn, triggers the decrease in oxidative stress through the activation of the intracellular antioxidative enzymes [90]. In our own studies, we observed the negative correlation between the Ado concentration in erythrocytes in vitro and in vivo and the lead concentration in blood [8, 9].

Free Radicals

Many authors have reported that the most important molecular mechanism of Pb toxicity is the production of free radicals [85, 91, 92]. Reactive oxygen species (ROS) react with cellular macromolecules (DNA, proteins, lipids), leading to impairments [67, 84, 85, 92, 93]. Erythrocytes are highly sensitive to lead-induced oxidative stress because of exposure of red blood cells to high O_2 concentrations, the ease with which hemoglobin may be autooxidized, peroxidation of membrane lipids resulting in further impairments of erythrocyte membrane and limited erythrocyte capability of repairing these impairments [91].

Free radical oxidation of unsaturated fatty (PUFA) may be induced in erythrocytes also by the presence of the border metals which enhance the peroxidation processes due to increased ROS production (Fenton's reaction) [52, 58, 94]. These changes are stimulated by iron, which is relatively easily released from transferine and ferrytine. Even a small increase in free Fe ions results in rapid ROS production [89].

Jedryczko [88] claimed that Pb^{2+} ions at the concentration of 0.01–0.4 mmol enhance Fe (II) and H_2O_2-induced peroxidation of membrane lipids. Lead ions, during the absence of iron or hydrogen peroxide ions, did not affect the peroxidation processes. The author suggests that although the Pb ions do not participate directly in the free radical oxidation and reduction reactions, they still change the cell membrane structure, limiting the mobility of membrane phospholipids and facilitate spread of free radical reactions [88].

Acknowledgements

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