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Original Research

The Effect of Environmental Contamination with Fluorine on the Concentration of Adenine Nucleotides in the Blood of Black and White Heifers

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Abstract

The purpose of this study was to examine the influence of environmental fluorine contamination on the adenylate nucleotides (AMP, ADP, AMP), total nucleotide pool (TAN), and adenylate energy charge (AEC) in the erythrocytes of heifers. The concentration of F in serum as well as ATP, ADP, AMP, TAN contents and AEC of red cells were also determined. The animals exposed to F^- (group A) came from farms situated about 2-3 km away from the Police chemical plant**.** The control group involved animals from an ecologically clean area (group B). In comparison with the control, heifers from a study group showed a significant decrease in ATP ($p \leq 0.001$) and ADP ($p \leq 0.01$), with a parallel increase in erythrocyte AMP ($p \leq 0.001$). The means serum F^- concentration was higher in exposed animals (6.5 μM, whereas in the control group it was 4.3 μM). The differences were statistically significant ($p \leq 0.05$). Both the TAN concentration in serum and the level of AEC in the group of heifers exposed to high F concentration was significantly lower than in the control group ($p \leq 0.001$).

Keywords: adenine nucleotides, adenine nucleotide pool, adenylate energy charge, fluorine, heifers

Introduction

Positive or negative effects of fluorine on organisms depend on the time and type of exposure. Due to its high electronegativity and high reactivity, fluorine does not occur in its elemental state. It binds with other elements, creating ion compounds (*e.g.* NaF, KF,CaF_2), covalent ones in a liquid or gas state (*e.g.* HF, SiF_4, JF_7), and covalent ones with high bond polarity (*e.g.* ZnF_2, MnF_2, CoF_2). Most of them are easily soluble in water, apart from aluminum, lithium, strontium, lead, magnesium and calcium fluorides [1].

The fluorine compounds are transmitted to the environment by industrial plants using materials consisting of fluorine (cryolite, fluorite, apatite), mainly aluminum mills, ironworks, and producers of phosphate fertilizers [2]. Long-term exposure of livestock, especially cattle, to the fluorides in food, water and the air, results in acute or chronic contamination. Metabolic disorders in systems, organs, tissues and cells, due to excess fluorine supply, lead to changes that are noticed usually quite late, including paresis, inhibition of lactation, tooth enamel discoloration, pain within long bones, bone decalcification, and hydrophobia [3-5].

Intake via the lungs and gastric system results in elevated blood fluorine levels. Yamamoto et al. [6] observed that 75% of blood fluorine is in the plasma, where it binds

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with proteins, whereas only 25% penetrates through the erythrocyte membrane. It seems that erythrocytes have special mechanisms that either protect against fluorides penetrating inside or are efficient in eliminating them [7].

As we know, the source of energy necessary to maintain the correct shape and functions of erythrocytes are adenine nucleotides (ATP, ADP, AMP), and 2.3-DPG, NADH and NADPH, produced by the process of glycolysis and pentose phosphate pathway [8, 9]. Their level is controlled by relevant enzymes; most of which are magnesium-dependent enzymes. Fluoride ions, due to their affinity to bivalent ions, are their inhibitors [10]. Thus fluoride ions were proved to inhibit hexokinase (EC 2.7.1.1), phosphate fructokinase (EC 2.7.1.11), phosphate pyruvate hydratase (EC 4.2.1.11), and pyruvate kinase (EC 2.7.1.40) [11, 12]. A decrease in enzyme activity indirectly affects the synthesis of energy compounds in erythrocytes. Some reports indicate that an increase in fluoride concentration in animal serum (both in laboratory conditions and environmental exposition) resulted in a decrease in ATP and ADP, adenine nucleotide pool (TAN) and the electric charge (AEC) [13-17].

The aim of this study was to determine the ATP, ADT and AMP concentrations, as well as TAN and AEC in erythrocytes of heifers chronically exposed to fluorides from their environment. Exposure was determined by measuring the fluoride concentration in the serum of the studied heifers and the control group.

Experimental Procedures

The study was performed during autumn and winter on 30 black and white heifers, from 12- to 15-months old. The 18 animals exposed to F^- came from private farms situated about 2-3 km southeast of the Police chemical plant in Poland. About 46.5 metric tons of fluorine is released into the atmosphere from this factory every year. In veterinary studies the cows demonstrated various degrees of fluorosis due to absorption of fluorine not only from the air or water (0.2 mM F), but also from F-contaminated fodder originating from polluted fields. The control group consisted of 12 heifers living in Korytowo, a relatively ecologically clean region 100 km from the contaminated area.

Blood was extracted in the morning hours from the exterior jugular vein into two separate test tubes (in different volumes). 7 mL of blood was collected into a heparinized test-tube (Heparinum – Polfa 250 JU) and 5 mL (for clotting) into a plastic test-tube. The blood samples were transported to the laboratory in an ice-filled thermos flask and analyzed immediately. The Packed Cell Volume (PCV) was determined from the heparinized blood samples with a microhematocrit centrifuge. Another aliquot was deproteinized with perchloric acid for measurement of the ATP, ADP and AMP content in the acid-soluble fraction of erythrocytes. This method was essentially that of Jaworek *et al.* [18] using the Biochemical Test Combination (Boehringer, Mannheim, Germany) with readings taken at 340 nm.

The adenine nucleotide concentration in erythrocytes was calculated using the value of the packed cell volume. Total adenylate nucleotides (TAN) and the adenylate energy charge (AEC) were calculated according to the formulas:

$$TAN = [ATP] + [ADP] + [AMP]$$

$$AEC = \frac{1}{2} \times \frac{[ADP] + 2[ATP]}{[ATP] + [ADP] + [AMP]}$$

Blood in the plastic tubes was centrifuged at 2000 rpm for 15 min, whereupon the serum was withdrawn and used to measure the F^- concentration (Marut) [19].

The results are expressed in SI units compared statistically with Student's t-test as well as the Mann-Whitney test (Statistica v.5.1 software). Spearman's rank correlation coefficient (r) was determined for F levels in serum vs. erythrocyte parameters in each study group. Significance levels of 0.05 and 0.01 were used to accept or discard hypotheses derived from the analytical data.

Results

The results of this study indicate prolonged exposure to fluorine results in significantly higher concentrations of F^- in the serum. In black-white race heifers from an area highly polluted with fluorine, the mean serum F concentration was 6.5 μM, whereas in the control group it was 4.3 μM. The differences were statistically significant ($p \leq 0.05$) (Table 1).

Table 1. Concentration of serum F [μM] in both groups of heifers.

Group	n	$\overline{X}$ [μM]	±SD
Study group	18	6.527*	0.751
Control group	12	4.3	0.241

n – number of animals in a group; $\overline{X}$ – mean arithmetic value; ± SD – standard deviation; *compared to control, $p \leq 0.05$.

Table 2. Correlation coefficient (r) between fluorine levels in blood serum and ATP content and adenylate energy charge (AEC) in heifer erythrocytes.

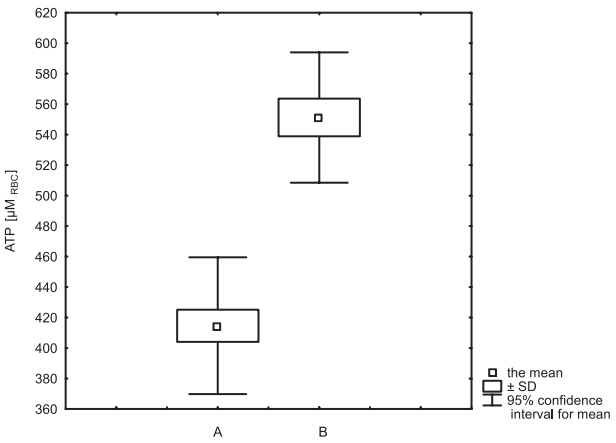
Dependent variable		Independent variable	
		ATP	AEC
Fluorine	Control group	- 0.2827	- 0.1697
	Study group	- 0.5082*	- 0.4904*

* – significant difference at $p \leq 0.05$.

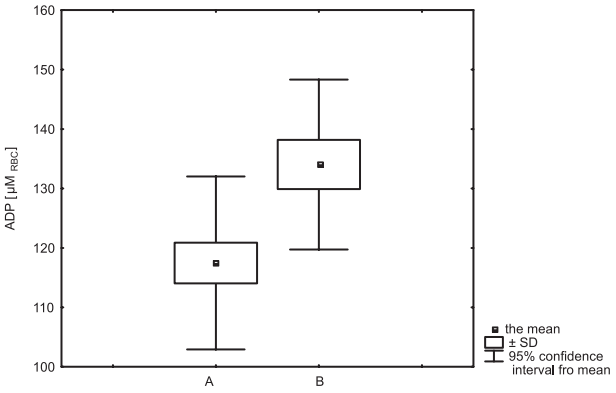
In the group of heifers exposed to high levels of F, a significant decrease in erythrocyte ATP ($p \leq 0.001$) and ADP ($p \leq 0.01$) compared to the control group were observed, with a simultaneous increase in erythrocyte AMP ($p \leq 0.001$) concentration (Figs. 1a-c). Additionally, both the TAN concentration in serum (sum of [ATP], [ADP] and [AMP]) and the level of AEC in the group of heifers exposed to high F concentration was significantly lower than in the control group ($p \leq 0.001$) (Figs. 2, 3).

A linear negative correlation ($r = -0.5082$) between the erythrocyte ATP concentration and the serum F concentration was observed in the heifers exposed to high concentration of fluorine. A similar correlation ($r = -0.4904$) was found between TAN level and serum F concentration in this group; no such relationship was observed in the control group (Table 2).

a).



b).



c).

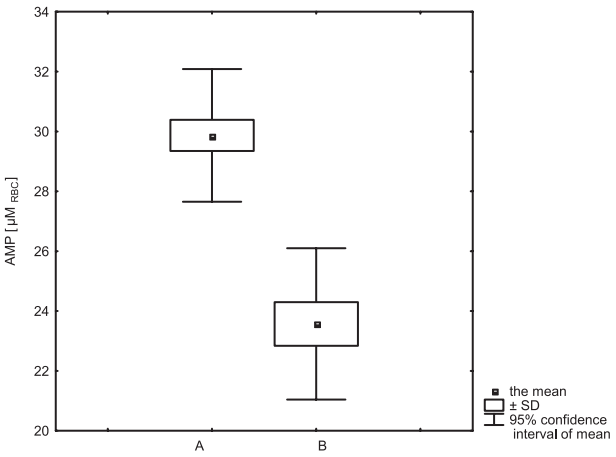


Fig. 1. a) ATP, b) ADP, c) AMP concentration in erythrocytes of heifers: A – study group, B – control group. Differences between groups statistically significant at $p \leq 0.001$ in case on a), c), and at $p \leq 0.01$ in b).

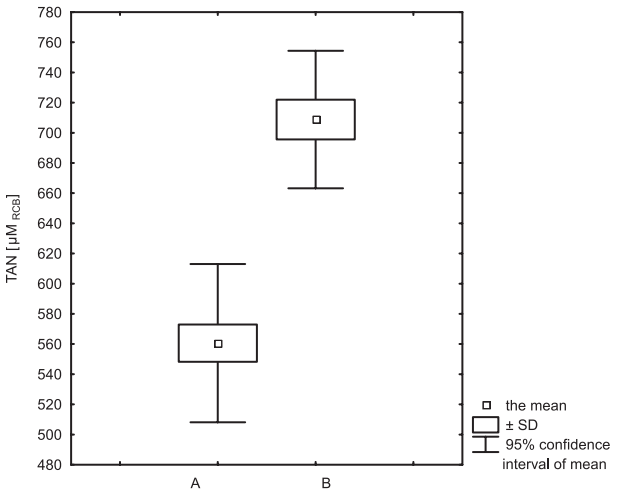


Fig. 2. Value of nucleotide pool (TAN) in erythrocytes of heifers: A – study group, B – control group. Differences between groups is statistically significant at $p \leq 0.001$.

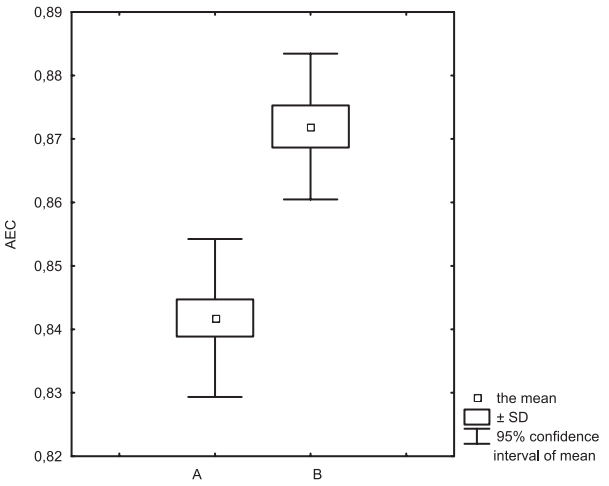


Fig. 3. Value of adenylate energy charge (AEC) in erythrocytes of heifers: A – study group, B – control group. Differences between groups statistically significant at $p \leq 0.001$.

Discussion of Results

An increase in environmental pollution from industrial emissions is drawing more and more attention. This includes an increased interest in the effect of fluorine on tissues, and in particular, the content of adenine nucleotides. Various reports have shown that exposure to fluorides affects ATP concentration in tissues [20-22, 13].

In this study, heifers' prolonged exposure to fluorides resulted in higher serum fluorine concentration than in the control group. It is interesting that the observed difference in F concentration between the study and control group was significant, while in our previous study on mature cows (3-4 years of age) of the same breed it was not significant [17]. It may be explained by fluorine's 'paradoxical effect', *i.e.* a lower dose or shorter exposure may result in a more acute disorder [23]. It indicates the existence of some adaptational and defensive mechanisms to excess fluorine accumulation in the system. One of these mechanisms may be fluorine intake and accumulation in the skeleton in the form of fluorapatite and magnesium fluoride [2].

Fluorine in blood binds with plasma proteins, mainly albumins, and in this form is not biologically active. Only the free ions in erythrocytes are capable of inhibiting the activity of magnesium-dependent enzymes in the process of glycolysis, and influencing the adenine nucleotide concentration, adenine energy charge and adenine nucleotide pool [24, 11, 6]. It is possible that the compensatory mechanisms are activated only after a certain contamination level is exceeded; this level is related to the time of exposure.

Physiological balance between the energy-producing and energy-consuming processes is lost during the presence of fluorine ions. Fluorine-induced inhibition of both pyruvate kinase activity and ATP-ase, observed in *in vitro* studies, suggests an inability to maintain the proper ATP concentration level [21, 25]. However, a catalytic function of pyruvate kinase leads to ATP consumption in the presence of fluorides [14]. In this reaction fluorides undergo phosphorylation in the presence of Mg^{2+}, creating fluoride phosphate**.** It is supposed that in this way, through active transport with ATP consumption, fluorides are excreted from erythrocytes.

In vitro studies also showed that the most acute fluorine-induced inhibition of glycolysis was with the lowest magnesium concentration [26]. Therefore, fluorine's negative effect can be neutralized by increasing magnesium in a diet. It could facilitate fluoride elimination and smooth the clinical symptoms accompanying magnesium and energy deficits. It is worth noticing that there may be some differences in Mg^{2+} concentration in erythrocytes and plasma between breeds and species. In black and white cows, Mg^{2+} concentration inside an erythrocyte is twice as high as in serum, a fact not reported in any other ruminants [27].

The balance between energy consumption and production (hydrolysis of ATP) is measured by AEC ratio [10]. A significant decrease in AEC, observed in the studied group of heifers, had a close connection with the decrease in TAN. The AEC decrease suggests inhibition of ADP phosphorylation into ATP.

The negative influence of fluorides on the ATP concentration is crucial in erythrocytes, where anaerobic glycolysis is the only way to produce energy. Lower ATP concentrations may lead to impairment of cation pump functions, (mainly Na+/K+ ATPase), an altered concentration gradient between the inside and outside of the cell, impairment of spectrin-actin cystoskeleton**,** loss of water and potassium ions (which consequently may change surface/volume ratio) and finally changes in the erythrocyte shape – from a biconcave one to an echinocyte [28].

Additionally, fluorides inhibit the activity of certain enzymes from the Krebs cycle [22, 13]. This involves both mitochondrial isocitrate dehydrogenase (NAD) and enzymes containing ferrum-sulphate centers: aconitate hydratase and succinate dehydrogenase, taking part in, *i.e*., oxidative phosphorylation [20]. It must be remembered that enzyme protein activity is also influenced by fluorine bonds with other ions (*e.g.* Al^{+3}, Be^{+2}) that are analogs of the phosphate group.

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