# Energy Metabolism of Erythrocytes in Lambs Chronically Exposed to Fluorine Compounds

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**Abstract**

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Studies were performed to test the effect of environmental fluorine compounds on the energy metabolism of erythrocytes in lambs (Merino × Kent). The concentration of fluorine in serum, ATP, ADP and AMP content in blood and erythrocytes, adenine nucleotide pool (TAN) and adenylate energy charge (AEC) of erythrocytes were determined. A significant decrease in ATP concentration and a significant increase in ADP concentration ($p \leq 0.05$) were observed compared to control group. The exposure of lambs to environmental fluorine compounds also caused a significant increase in the content of fluorine in serum.

Moreover, a negative linear correlation ($r = -0.836$) between the erythrocyte ATP concentration and the serum fluorine ion concentration was found. AEC level also correlated linearly ($r \leq -0.949$) with the concentration of fluorine ions in serum.

These observations suggest that exposure of lambs to environmental fluorine compounds resulted in impaired energy metabolism of their erythrocytes.

*Adenine nucleotides, adenine nucleotide pool, adenylate energy charge, fluorine, blood*

Fluorine is known for its exceptional biological activity (Guminska 1994). Chronic environmental exposure of animals to fluorine compounds results in bone fluorosis and accumulation of the element in tendons, articular capsules and muscles (Guminska 1981; Pirog and Socha 2000). The accumulation of fluorine in the body continues during the whole lifespan and constitutes a serious threat to health (Mokrzynski et al. 1994; Chlubek et al. 1994; Machoy 1995). In animals, tenderness of long bone epiphyses, pareses, demineralisation and bone fragility have been observed. Apart from the relatively late anatomical lesions, fluorine is responsible for metabolic disorders in systems, organs, tissues and individual cells (Chlubek et al. 1995; Ignacak and Guminska 1991; Machoy 1995). Fluorine enters the bloodstream through the intestines and lungs. According to Guminska (1990), approx. 75% of fluorine appears in plasma, the remaining part in erythrocytes. Apparently, red blood cells possess a special mechanism protecting them against entry or facilitating elimination of fluorine (Korkmaz 2000). Yamamoto et al. (1989) studied the distribution of this element in blood and found that fluorine exists as a free ion, in inorganic and organic compounds. It also binds to plasma proteins, mainly albumin, and as such is biologically inactive (Guminska 1981). Free ions are the active form of fluorine, inhibiting approximately 70 enzymes (Guminska 1994), among them magnesium-dependent transport enzymes (Grabowska et al. 1991; Morris 1992; London and Gabel 1995) and several metabolic pathways (Guminska 1985; Machoy 1987) in red blood cells.

Fluorine is a halogen with the lowest mass and ion radius and the highest electronegative potential and reactivity (Wakselman 1999). It may act directly on the enzyme protein, leading to structural changes through disruption of hydrogen bonds, or indirectly by

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removing cations necessary for enzyme activity (Machoy 1987). Studies on the influence of fluorine compounds on magnesium-dependent enzymes have convincingly shown that fluorine ions inhibit the activity of enzymes participating in glycolysis and the pentose cycle in red blood cells (Guminska 1981, 1985, 1994), indirectly affecting their adenine nucleotide pool.

Following this line of reasoning, we investigated the concentration of ATP, ADP and AMP in erythrocytes, to determine the adenylate energy charge of these cells and relate the results to the concentration of fluorine in serum. We used lambs bred near a major chemical plant in Police and thus chronically exposed to fluorine.

## Materials and Methods

### Animals

The study was performed during autumn and winter 1999 in 24 lambs of Merino × Kent strain aged approx. 12 months. The animals were bred in farms located within 2-3 km from the Police Chemical Plant. This plant annually releases some 46.5 tons of fluorine compounds into the atmosphere (Zabłocki 1998). The animals were fed hay and silage of corn and sugar beet leaves. The fodder originated from fields polluted with fluorine compounds. Veterinary examination of the animals did not reveal any signs of fluorosis. Nevertheless, the lambs were apparently weaker, weighed less and consumed less food than controls.

The control group consisted of 15 healthy lambs of the same strain, bred at least 70 km south-west of Police. They were fed the same type of fodder coming from unpolluted fields.

### Analytical procedure

Blood was obtained from the jugular vein between 08.00 and 09.00 h. A 7 ml sample was transferred to a heparinized (250 IU heparin from Polfa, Poland), and 5 ml to a standard plastic test tube. Blood was delivered to the laboratory in ice flasks and immediately analyzed.

### Biochemical analyses

Heparinized blood served to measure hematocrit. Following deproteinization with perchloric acid, ATP, ADP and AMP were measured in the acid-soluble fraction of erythrocytes with test kits (Biochemica Test Combination, Boehringer Mannheim). From absorbance differences determined according to the Schmidt method (1974) the adenine nucleotides concentration (μmol/l whole blood) in whole blood was calculated. The adenine nucleotides concentration in erythrocytes was calculated using hematocrit. The value of the nucleotide pool (TAN) and the adenylate energy charge (AEC) was calculated according to the formulas (Atkinson D.E. 1968):

$$\text{TAN} = (\text{ATP}) + (\text{ADP}) + (\text{AMP}); \quad \text{AEC} = \frac{1}{2} \times \frac{(\text{ADP}) + (\text{ATP})}{(\text{ATP}) + (\text{ADP}) + (\text{AMP})}$$

Blood in plastic test tubes was centrifuged for 15 min at the RCF value 720 *g* and fluorine level in serum was determined according to Maruta's method (1978).

### Statistical analysis

The results were expressed in SI units. Statistical analysis was performed using Student's *t*-test (Statgraphics v. 50 software). Pearson's correlation coefficient (r) was determined for fluorine levels in serum vs. erythrocyte energy parameters in each study group. The significance level of 0.05 was implemented to serve as a criterion for approving or discarding hypotheses for drawing the conclusion, relying on statistical analytic data.

## Results and Discussion

Adenine nucleotides play a key role in the energy metabolism of cells. Levels of ATP, ADP and AMP reflect the rate of energy transformations in mature red blood cells, fueled mainly by glycolysis and the pentose cycle (Kwiatkowska 1989). The adenine nucleotide pool necessary for normal activity of erythrocytes remains under the control of an elaborate set of enzymes (Ataullakhonov et al. 1981), which includes three magnesium-dependent regulatory enzymes: hexokinase (EC.2.7.1.1), phosphofructokinase (EC.2.7.1.11) and pyruvate kinase (EC.2.7.1.40), all inhibited by fluorine ions (Machoy 1987; Guminska 1994). Minor shifts in the level of allosteric activators and inhibitors may grossly affect the rate of enzyme reactions, among them glycolysis, leading indirectly to changes in the level of ATP, ADP and AMP (Dabrowska 1997) and in the adenylate energetic charge of erythrocytes (Atkinson 1968).

Our lambs were exposed to fluorine compounds during approximately 12 months. The serum content of fluorine in the study group was found to be significantly higher (157.1 ± 12.94 μM) than in controls (116.3 ± 15.35 μM). This was accompanied by reduced ATP levels in erythrocytes and elevated ADP levels in blood ($p \leq 0.05$) (Table 1). Veterinary examination revealed no signs of fluorosis in the study animals. The inhibitory effect of fluorine on magnesium-dependent enzymes found by other authors (Guminska 1981; Machoy 1987) was confirmed in our study in the form of significantly lower levels of ATP. Furthermore, serum levels of fluorine in animals exposed to fluorine correlated inversely with erythrocyte levels of ATP and adenylate energetic charge of these cells (Table 2). The energy charge is a useful marker of shifts between consumption of energy stored in ATP and its production in metabolic processes (Atkinson 1968). The adenine nucleotide pools in both groups of animals did not differ significantly (Table 3). Moreover, changes in the energy metabolism of erythrocytes are frequently associated with disorders of mineral balance (Kedryna et al. 1991; Hłynczak et al. 1980; Guminska and Skowron-Sula 1985).

Table 1
Concentration of adenine nucleotides (μmol/l) in erythrocytes and whole blood of lambs chronically exposed to fluorine compounds (mean ± SD)

| Group | ATP | | ADP | | AMP | |
|---|---|---|---|---|---|---|
| | Erythrocytes | Whole blood | Erythrocytes | Whole blood | Erythrocytes | Whole blood |
| Exposed | 278.81* | 86.43 | 132.37 | 46.33* | 19.87 | 6.16 |
| (n=24) | 39.773 | 12.830 | 23.334 | 7.527 | 6.882 | 2.216 |
| Control | 292.87 | 90.79 | 124.55 | 38.61 | 20.81 | 4.65 |
| (n=15) | 31.824 | 30.039 | 22.274 | 14.176 | 3.441 | 2.844 |

* - significant difference ($p \leq 0.05$)

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

| Dependent variable | | Independent variable | |
|---|---|---|---|
| | | ATP | AEC |
| Fluorine | Control group | -0.2645 | -0.0046 |
| | Exposed group | -0.8364* | -0.9487* |

* - significant difference ($p \leq 0.05$)

Table 3
Values of adenylate energy charge (AEC) and nucleotide pool in erythrocytes of lambs chronically exposed to fluorine compounds (mean ± SD)

| Group | AEC | Nucleotide pool (μmol/l RBC) |
|---|---|---|
| exposed (n = 20) | 0.8004 ± 0.0543 | 431.05 ± 49.989 |
| control (n = 15) | 0.8104 ± 0.0543 | 438.23 ± 51.480 |

Qualitative and quantitative distribution of electrolytes in the red blood cell varies, depending on species and strain of animal. For example, sheep erythrocytes differ according to levels of sodium and potassium. Two inheritable forms have been described. HK (high potassium) cells have high levels of potassium and low levels of sodium, while the inverse is true of LK (low potassium) cells (Baranowski 1998). HK lambs usually have a higher body mass than their LK counterparts (Atroshi 1979) and are more valuable as reproductors (Kmieć 1989; Lipecka et al. 1994). Other mammals, including cats, dogs and cows belong

to the LK type (De Meydonca et al. 1970). Differences between animal species concerning magnesium content in erythrocytes and plasma have been reported. Sheep usually have low levels of this element in red blood cells and plasma (Torrance 1972) and in consequence are more susceptible to fluorine than other mammals. Guminska (1985) has observed a strong correlation between magnesium content in erythrocytes and potency of fluorine to inhibit various enzymes. The antagonism between fluorine and magnesium is well known and has been traced *in vivo* to the loss of magnesium from erythrocytes in the presence of fluorine ions (Marier 1980b; Stachurska and Guminska 1991; Guminska 1994).

Reduced levels of ATP in erythrocytes have been observed in adults and children residing in environments contaminated with fluorine (Hłynczak et al. 1980). Changes in levels of adenine nucleotides depend not only on the exposure period but also on the dietary content of magnesium (Guminska 1985; Guminska 1994). Our results concerning ATP, ADP and AMP levels, as well as the adenylate energy charge in lambs seem to be the consequence of elevated levels of fluorine in serum. As a countermeasure against the negative effects of fluorine on energy metabolism (Guminska 1985), the fodder fed to animals in contaminated regions should be enriched with magnesium.

Lambs have relatively low levels of magnesium in plasma and erythrocytes and in consequence are more susceptible to toxic effects of fluorine.

Exposure of animals to fluorine compounds leads to abnormalities in energy metabolism of erythrocytes, presumably due to the inhibitory effect of fluorine on magnesium-dependent enzymes, catalyzing the process of glycolysis.

**Energetický metabolismus erytrocytů jehňat vystavených chronickému působení sloučenin fluóru**

Studie byla zaměřena na zjištění účinků sloučenin fluóru pocházejících ze životního prostředí na energetický metabolismus erytrocytů jehňat (Merino x Kent). Byla stanovena koncentrace fluóru v séru, obsah ATP, ADP a AMP v krvi a erytrocytech, pool adeninových nukleotidů (TAN) a adenylátový energetický zdroj (AEC) erytrocytů. V porovnání s kontrolní skupinou bylo zjištěno signifikantní snížení koncentrace ATP a signifikantní zvýšení koncentrace ADP ($p < 0.05$). Kromě toho vystavení jehňat působení environmentálních sloučenin fluóru způsobilo také signifikantní zvýšení obsahu fluóru v krvním séru. Mimo to byla nalezena negativní lineární korelace ($r = -0.836$) mezi koncentrací ATP v erytrocytech a koncentrací fluoridových iontů v séru. Také úroveň AEC byla v lineární korelaci ($r = -0.949$) s koncentrací fluoridových iontů v séru. Z výsledků vyplývá, že účinkem sloučenin fluóru pocházejících ze životního prostředí dochází ke snížení energetického metabolismu erytrocytů jehňat.

**References**

ATAULLAKHONOW, FJ, VITVITSKY, VM, ZHABOTINSKY, AM, PICHUGIN, AV, PLATONOVA, OV, KHOLODENKO, BN, EHRLICH, LJ 1981: The regulation of glycolysis in human erythrocytes. Eur J Biochem **115:** 359-365

ATKINSON, DE 1968: The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry **7:** 4030-4034

ATROSHI, F 1979: Phenotypic and genetic association between production/ reproduction traits and blood biochemical polymorphism characters in Finnsheep. Agric Res Centr Inst Anim Breed. Finland: Dissertation. Agr. 74 p.

BARANOWSKI, P. 1998: Selected biochemical indicators of lamb blood serum in relation to the erythrocyte potassium level. Animal Science Papers and Reports 1: 25-33

CHLUBEK, D, MOKRZYNSKI, S, MACHOY, Z, SAMUJLO, D, WEGRZYNOWSKI, J 1994: Fluoride concentration in mother and fetus. I. Placental transport of fluorides. Ginekol Pol **65**: 611-615

CHLUBEK, D, MOKRZYNSKI, S, MACHOY, Z, OLSZEWSKA, M 1995: Fluorides in the body of the mother and the fetus. III. Fluorides in amniotic fluid. Ginekol Pol **66**:614-617

DABROWSKA, A 1997: Red cell pyruvate kinase maybe control of oxygen delivery from erythrocyte. Post Hig Med Dosw **51:** 305-318

DeMENDONCA, MC, SCHWARTZ, K, TERRIER, E 1970: Sodium, potassium and osmolarity of human and canine erythrocytes. Comp. Biochem. Physiol. **34**: 147-151

GRABOWSKA, M, GUMINSKA, M, IGNACAK, J 1991: Inhibitory effect of environmental pollutants on erythrocyte membrane ATPase activity in humans. Folia Med Cracov **32**: 103-110

GUMINSKA, M 1981: Biochemical mechanisms of fluorine action on living organism. Folia Med Cracov **23:** 305-321

GUMINSKA, M, SKOWRON-SULA, M 1985: The effect of magnesium and fluoride concentrations on erythrocyte glycolysis in vitro. Folia Med Cracov **26:** 35-41

GUMINSKA, M 1990: Biochemical mechanisms of environmental fluoride action. Arch Ochr Srodowiska **3-4:** 105-115

GUMINSKA, M 1994: Influence of fluorides on energy metabolism in vitro and in vivo and related biological effects. VI Symp. Metabolism of Fluorine, Szczecin, p. 9-11

HLYNCZAK, AJ, KOSMIDER, K, ADAMOWICZ, A, FOKT, M, GIEBAS, K, URBANSKA, A 1980: Energetic erythrocyte pathways in persons exposed to the action of fluorine compounds according to age and length of service. Biul Inst Med Morsk **31:** 61-67

IGNACAK, J, GUMINSKA, M 1991: Fluorides in urine of the population living in polluted environment. Folia Med Cracov **32:** 89-95

KEDRYNA, T, MARCHUT, M, GUMINSKA, M 1991: Changes in carbohydrate and energy metabolism during biochemical studies of Chorzow inhabitants, chronically exposed to environmental pollution. Folia Med Cracov **32:** 103-110

KMIEC, M 1989: Niektore cech użytkowe i biochemiczne krwi u owiec długowełnistych o rożnych fenotypach poziomu potasu. Rocz Nauk Rol **105**: 31-42

KORKMAZ, O 2000: In vitro effects of sodium fluoride and sodium dichromate on dynamic properties of human erythrocyte membrane. Biophys Chem **83:** 111-120

LIPECKA, C, PIETA, M, GRUSZCZECKI, T 1994: The potassium level in the blood of sheep and their productivity. J Anim Feed Sci **3:** 89-96

LONDON, RE, GABEL, SA 1995: Fluorine-19 NMR studies of glucosyl fluoride transport in human erythrocytes. Biophys J **69:** 1814-1818

MACHOY, Z 1987: Biochemical mechanisms of fluorine compounds action. Folia Med Cracov **28:** 61-81

MACHOY, Z, DABKOWSKA, E, SAMUJLO, D, OGONSKI, T, RACZYNSKI, J, GEBCZYNSKA, Z 1995: Relationship between fluoride content in bones and the age in European elk (Alces alces L.) Comp Biochem Physiol C Pharmacol Toxicol Endocrinol **111:** 117-120

MARIER, JR 1980: Observations and implications of the /Mg vs F/ interrelations in biosystems: a review. Proc Finn Deut Soc **76:** 82-92

MARUTA, 1978: Prosta fotometryczna metoda oznaczania steżenia fluoru w surowicy krwi i moczu. Diagn Lab **14:** 253-262

MOKRZYNSKI, S, CHLUBEK, D, MACHOY, Z, SAMUJLO, D 1994: Fluoride in the organism of mother and fetus. II. Fluoride cumulation in the organism of the fetus. Ginekol Pol **65:** 678-681

MORRIS, MB, MONTEITH, G, ROUFOGALIS, BD 1992.: The inhibition of ATP-dependent shape change of human erythrocyte ghosts correlates with an inhibition of $(Mg^{2+})$-ATP activity by fluoride and aluminofluoride complexes. J Cell Biochim **48:** 356-366

PIROG, B, SOCHA, M 2000: Positive and negative effects of fluorine in the human organism. The source of fluorine in the environment. Med Pracy **51:** 75-78

SCHMIDT, E 1974: Methoden der enzymatischen analayse Chemie, Mannheim, 125 p.

STACHURSKA, MB, GUMINSKA, M 1991: Magnesium in the blood and urine of the population living in polluted environment of Chorzow. Folia Med Cracov **32:** 89-94

TORRANCE, JD 1972: Erythrocyte 2,3-DPG in various mammalian species. II nd Intern. Symp. : Erythrocytes, Thrombocytes, Leukocytes, Vienna, p. 161-164

WAKSELNAM, C 1999: Fluorinated organic compounds: synthesis and biological applications. Ann Pharm Fr **57:** 108-115

YAMAMOTO, G, YOSHITAKE, K, SATO, T, KIMURA, T, AANDO, T 1989: Distribution and forms of fluorine in whole blood of human male Anal Biochem **182:** 371-376

ZABLOCKI, Z 1998: Changes of the fluorine contonent in some components of environment in the area affected by „Police" Chemical Works emissions in 1977-1996. VII Symp. Metabolism of Fluorine, Szczecin,p. 16-23