SPECIAL SUPPLEMENT

ADDITIONAL ABSTRACTS OF PAPERS PRESENTED AT THE XXIIIRD CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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Content of fluorides soluble and insoluble in potassium hydroxide in superficial layers of enamel in adults regularly using fluoride-supplemented toothpaste

The reaction of fluoride ions with enamel apatites produces fluorohydroxyapatites and calcium fluoride. Relative quantities of these products depend on the fluoridation method, concentration of fluoride in the oral cavity, type of fluorine preparation, pH and time. The type of compound formed on enamel surface or incorporated into its crystalline structure is of prime importance for caries prevention.

The aim of this work was to determine the content of calcium fluoride and fluorohydroxyapatites in three superficial layers of enamel in adults regularly using fluoride toothpaste. The premolar teeth studied were extracted from patients who used fluoride toothpaste twice daily and for at least 12 months prior to the study did not use any other fluoride preparation.

Enamel samples were obtained using acid biopsy with 0.5 M perchloric acid for 30 s. Calcium fluoride was removed from enamel surface and pores by 24 h incubation in 1 M potassium hydroxide. Fluoride content was determined using gas chromatography (GC-FID). Calcium was measured using flame ASA.

A logarithmic distribution of fluorides in enamel depending on depth of digestion was obtained. Furthermore, an inverse correlation between the depth of digestion and fluoride content in the form of fluorohydroxyapatites and calcium fluoride was observed. Calcium fluoride represented over 50% of total fluorides in the external enamel layer of approx. 6 μm. Removal of calcium fluoride from enamel surface and pores did not significantly affect the solubility in acid of superficial layers of enamel, suggesting that one of the factors determining enamel solubility in acids is fluorohydroxyapatite. A higher content of fluorohydroxyapatite in the outermost layer of enamel may be explained by mineral exchange cycles taking place in the presence of fluoride ions and leading to progressive increase in enamel resistance to acids.

The present results indicate that fluorides in toothpaste are one of the factors responsible for post-eruption protection of enamel surface against caries, as reflected by the highest content of fluorides in the outermost layer of enamel.

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Fluoride levels in atheromatous plaques from carotid arteries

A dysequilibrium in the concentrations of elements in the vascular wall may stimulate atherogenesis. Changes in the concentration of calcium, magnesium, copper, zinc and fluoride (non-physiological ion) adversely affect lipid metabolism and activity of some enzymes in the vascular wall.

It was observed in the sixties that fluoride accumulates in the arterial wall but until now its presence in the atheromatous plaque has not been studied. It is also unknown whether calcification stimulates the deposition of fluoride.

We have measured the level of fluoride and calcium in atheromatous plaques from patients with severe atherosclerosis of carotid arteries. The plaque was cleaned free of erythrocytes, dissolved in nitric acid and extracted with benzene. Measurements were done with a gas chromatograph equipped with a 2.0-m column (20% DC 200 silicon oil on Chromosorb P, FID detector temperature 270°C, injector temperature 220°C, oven temperature 85°C). Calcium was measured using a Philips PU 9100X atomic absorption spectrometer.

It was found that fluoride accumulates in atheromatous plaques, like in the arterial wall, and that calcified plaques have a higher concentration of this ion.

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Fluorine content in osteoporotic and normal bones

The content of fluorine was established in 20 bones from healthy individuals who did not report any former treatment for osteoporosis. The results were compared with 20 osteoporotic bones collected from patients with fracture of the femoral neck. All subjects were residents of Szczecin or surroundings and their age ranged from 40 to 80 years (mean 67 years). Fluorine was measured in the compact and trabecular layers using a potentiometric method.

We found: (1) elevated mean content of fluorine in the trabecular layer in both groups, (2) elevated content of fluorine in the compact and trabecular layers in the healthy group ($p < 0.001$), (3) no correlation between age and fluorine content.

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Content of basic minerals in normal bones – preliminary report

We have examined bone samples from 20 patients without any history of bone pathology. Their age ranged from 40 to 80 years (mean 60.2 years). Samples were cleaned free of any other tissue, delipidated and dried. The spongy

and cortical layers were separated and pulverized. Fluorine was measured using a potentiometric method. Calcium, magnesium and zinc were determined with atomic absorption.

Results: (1) mean calcium content in the cortical and spongy layers was 271.53 and 292.21 mg/g, respectively, (2) mean magnesium content in the cortical and spongy layers was 2.97 and 2.88 mg/g, respectively, (3) mean zinc content in the cortical and spongy layers was 0.47 and 0.38 mg/g, respectively, (4) mean fluorine content in the cortical and spongy layers was 757.38 and 1094.15 ppm, respectively. The difference in the case of fluorine was statistically significant ($p < 0.001$).

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Fluoride levels in structural elements of chicken femur depending on age

The femur of chickens (n=100) of the Starbro Hybro Iloman strain was studied between day 1 and 50 of life. Four structures were examined: compact bone, spongy bone, bone marrow and articular cartilage. Fluoride levels were measured with a Chrom-5 gas chromatograph equipped with a 2.0 m metal column (ID 3.0 mm, 20% DC 200 silicon oil on Chromosorb P, FID detector temperature 270°C, injector temperature 220°C, carrier gas (nitrogen) flow rate 20 cm^3/min). The chickens were divided into 5 age groups.

Statistically significant differences in F content were found between the structural elements ($p < 0.001$), as well as between age groups ($p < 0.001$). During the first ten days of life the highest content of fluoride was found in compact bone. It continued to increase until the fourth decade and thereafter the level stabilized. A similar pattern was observed for spongy bone. Levels in bone marrow were lower than in compact or spongy bone and remained essentially unchanged. The lowest levels were observed in cartilage and continued to fall during the observation period.

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Circulation of fluorides in the life cycle of the edible snail *Helix aspersa maxima*

The aim of this work was to study the accumulation of fluoride during the life cycle of the edible snail *Helix aspersa maxima* under controlled culture conditions, in order to check the hypothesis that this can serve as a marker of environmental contamination with F compounds. Furthermore, such informa-

tion is important to ascertain that the snails meet criteria established for healthy food diets.

The culture was carried out for five months in three groups. Group A (control) was given a standard pulverized chow, which was supplemented with NaF at a dose of 1.5 (group B) or 150 (group C) mg F^-/l. Measurements were done in tissues of mature snails, shells, eggshells, freshly hatched snails and excrements of mature snails collected during the last day of experiment. F levels were established using an ion-selective electrode.

Preliminary results: toxic fluorine compounds are principally removed with excrements, but are also incorporated into the shell and eggshell; high F levels in the chow do not affect the viability of snails (as represented by mortality and fertility); *Helix aspersa maxima* demonstrates a high tolerance to fluoride load. There were no significant differences between group A (control) and B as to F levels in shells, eggshells, excrements and no differences between all groups in the case of freshly hatched snails. Significant differences were found between groups A and B on one side, and C on the other; increased F levels in chow seem to be without influence on the offspring (no significant differences in F levels between freshly hatched snails in all groups).

The high tolerance of *Helix aspersa maxima* to fluoride load makes this species a valuable marker of F content in the environment. For this reason, however, consumption of the snail by humans should be controlled in order to avoid chronic fluoride intoxication.

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The influence of sodium fluoride on the activity of alkaline phosphatase isoenzymes in rats

Alkaline phosphatase (ALP) activity in serum is composed of isoenzymes originating from different tissues. Changes in total activity may be attributed to the release of isoenzymes, in particular from the small intestine, bones and liver.

The aim of this work was to study the influence of sodium fluoride on the total activity of alkaline phosphatase and its isoenzymes in serum, liver, small intestine and long bones of rats.

Six-month-old male Wistar rats on a standard chow were divided into two groups of 10 animals each. The study group was exposed to 40 ppm NaF in drinking water. After 10 weeks the animals were anesthetized with ketamine before collection of blood and organs for further examination. ALP activity was determined with a kinetic spectrophotometric method at 410 nm, using p-nitrophenylphosphate as substrate. The activity of individual isoenzymes was measured with specific inhibitors: 10 mM L-phenylalanine for intestinal, and 3 M urea for bone isoenzyme. Samples were also incubated at 56° C for 15 minutes to inhibit the bone isoenzyme. Additionally, isoenzymes were separated

using agarose gel electrophoresis (HYDRAGEL ISO-PAL and HYDRAGEL ISO-PAL "Affinity" from Sebia). Activity was expressed in international units (U) per gram wet weight.

Initial measurements in some of the animals gave the following isoenzyme activities: liver 0.87, intestine 15.9, and bone 0.58 U/g in controls, and 0.73, 8.82 and 1.82 U/g, respectively, in the study group. The ratio of liver to bone activity was 1.45 in controls and 0.4 in rats exposed to NaF. No significant differences in the case of total serum activity were observed between groups.

Conclusions: Fluoride increases the activity of ALP in bone and reduced the activity in liver and intestine. These changes are not reflected in total serum activity. It seems justified to examine the activity of individual isoenzymes in serum in order to gain a better insight into the effects of fluoride on tissue levels of ALP.

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Fluorine and magnesium content in enamel of permanent human teeth with and without abrasion

The development and mineralization of enamel depends on a balanced uptake and loss of magnesium and fluorine. Both elements affect the activity of acid phosphatases which catalyze reactions leading to the formation of apatite crystals. Magnesium influences the physicochemical stability of apatite crystals and inhibits precipitation and growth of calcium apatites, in this way influencing the process of amelogenesis. Increased content of magnesium together with lower content of fluorine is one of the risk factors in pathological abrasion.

The aim of this study was to measure the content of fluoride and magnesium in superficial layers of enamel and in total enamel of teeth with abrasive changes. Teeth with and without abrasion were extracted from adults aged 40-60 years, residing in Szczecin. Microsamples of superficial enamel were obtained using acid biopsy. Fluoride and magnesium in enamel were measured after separation of enamel from other structures and dissolution in 1M perchloric acid. Fluoride content was determined using gas chromatography (GC-FID). Magnesium was measured using flame ASA.

A significantly higher content of magnesium and lower content of fluoride was observed in superficial layers of enamel from teeth with abrasion. Such teeth were also more susceptible to acid digestion, as evidenced by greater mass of enamel digested. The content of fluoride and magnesium in total enamel revealed no statistically significant differences between teeth with and without abrasion (in teeth with abrasion the fluoride content was lower and magnesium content higher, but the differences were not significant). The results suggest that the chemical structure of superficial layers of enamel in di-

rect contact with the oral cavity is one of the factors responsible for the susceptibility of enamel to abrasion.

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The influence of fluoride ions on antioxidative enzymes and malondialdehyde levels in human erythrocytes

The activity of antioxidative enzymes in erythrocytes, such as superoxide dismutase, glutathione peroxidase, and catalase plays a decisive role in the maintenance of cellular integrity. Their concerted metabolic action protects red blood cells against free radicals, most notably O_2^- which is formed during autoxidation of hemoglobin to methemoglobin. They also prevent the peroxidation of membrane lipids resulting in the production of malondialdehyde and lysis of erythrocytes.

Fluoride ions interact with a range of proteins exhibiting various biological functions, usually leading to inhibition, sometimes activation of enzyme activity. They can bind both with the active center and positively charged distant domains. They easily pass from the blood into cells, but their concentration in plasma is usually higher than in erythrocytes.

We have incubated erythrocytes from 10 healthy subjects in NaF solutions ranging from 0.25 to 2.5 mmol/l. Subsequently, the activity of superoxide dismutase, glutathione peroxidase and catalase, and concentration of malondialdehyde was determined and the results were compared with erythrocytes incubated without NaF.

Results: (1) erythrocytes incubated in 2.5 mmol/l NaF demonstrated significantly ($p = 0.05$) lower levels of malondialdehyde, (2) erythrocytes incubated in 0.25 mmol/l NaF demonstrated significantly ($p = 0.03$) higher activity of catalase, (3) incubation in NaF did not affect the activity of superoxide dismutase and glutathione peroxidase.

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Content of collagen, fluoride, phosphate and calcium in bones of rats exposed to sodium fluoride

Sodium fluoride in used for the treatment of osteoporosis. We have examined its influence on the organic (total and glycated collagen) and inorganic (fluoride, phosphate, calcium) constituents of bone in rats exposed to 50 and 100 ppm NaF in drinking water during 4 months. The control group was given

water without NaF. Collagen was also fractionated using SDS electrophoresis on polyacrylamide gel.

The results show a correlation between the level of F in water and bones ($p < 0.001$). No such correlation was confirmed in the case of collagen, phosphate and calcium ($p > 0.1$).

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Content of fluorides and other microelements in enamel of permanent teeth with active caries – an *in vitro* study

Enamel is composed of apatites, the most important one being hydroxyapatite. Some of the sites in the crystalline network normally occupied by calcium atoms, hydroxyl or phosphate groups may remain empty or may contain other elements, like F, Mg, Pb, Sr, Zn, introduced during treatment and prophylaxis, or as a result of environmental pollution.

The chemical composition and structure of enamel determines the susceptibility of hard dental tissues to caries. Fluorine is known for its anti-caries activity confirmed in numerous clinical and experimental studies. Contrary to this, the influence of lead, zinc and magnesium on the susceptibility of hard dental tissues remains equivocal. Several studies have suggested a pro-caries effect, others failed to find any change. Some reports indicate that lead neutralizes the protective action of fluoride.

The aim of this work was to examine the content of fluorine, zinc, magnesium and lead in the enamel of teeth with and without caries. Enamel was obtained from teeth extracted in adults, residents of Szczecin, regularly using fluoride-supplemented toothpaste. Fluoride content was determined using gas chromatography (GC-FID). Zinc, magnesium and lead were measured using flame ASA.

A significantly ($p \leq 0.05$) higher content of fluorides in enamel of teeth without caries was observed. Enamel of teeth with caries contained more lead but the difference was not statistically significant. No correlation between content of lead and susceptibility to caries was found. The high content of lead in enamel may be explained by environmental pollution. No statistically significant differences as to content of magnesium and zinc were revealed between both groups of teeth.

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Intensity of caries and content of fluorides and magnesium in enamel of permanent teeth in children residing in Szczecin and Białystok

Differences in industrialization of the region of Szczecin and Białystok are reflected by varying environmental pollution levels. Compounds released into the atmosphere by industrial plants may affect processes taking place in human enamel. In this context, the chemical composition of enamel is one of the decisive factors in the susceptibility to caries.

The aim of this study was to compare the intensity of caries in permanent teeth of children residing in Szczecin and Białystok and to evaluate the content of fluoride and magnesium in enamel. 60 children (30 boys and 30 girls), aged 12, 13 and 14 years, were enrolled. Caries intensity was represented by mean PUW and its components. Fluoride and magnesium content was determined in healthy teeth extracted during orthodontic treatment. Microsamples of enamel were obtained using acid biopsy. Fluoride content was determined using gas chromatography (GC-FID). Calcium and magnesium were measured using flame ASA.

Caries was more intense (PUW = 6.78) in children residing in Białystok than in Szczecin (PUW = 6.00), but the difference was not statistically significant. Significantly higher content of magnesium and lower content of fluoride in superficial layers of enamel was observed in children from the non-industrialized Białystok. The fluoride content in enamel of children residing in Szczecin was 58.30 mmol/kg higher than in Białystok. These differences may be explained by the loss of magnesium in the form of insoluble MgF_2 due to a higher level of F in the environment. Likewise, emitted SO_2 is oxidized to SO_3 and binds magnesium contained in soil to form water-insoluble sulphates. Enamel of children residing in Szczecin was more resistant to acid digestion, as reflected by smaller depth of the digested layer. Presumably, calcium and fluoride in the environment favour the mineralization of dental tissues and reduce caries. This suggestion is supported by a lower intensity of caries in children residing in Szczecin. Fluorides are one of the factors protecting the enamel against caries.

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Content of fluoride and magnesium in superficial layer of human enamel with erosion – an *in vivo* study

The enamel of human teeth is composed of hydroxyapatites and is characterized by apparent lack of activity on its surface. Physiological processes in

the oral cavity may influence the demineralization and remineralization of hard tissues of the teeth. Calcium atoms, hydroxyl and phosphate groups occupying some sites within the crystalline network of hydroxyapatites may be replaced by other elements. The result is hydroxyapatites with low calcium content, and the formation of carboxyapatites, chloroapatites, fluoroapatites and other forms of calcium phosphate. Acids in the diet or formed in the dental plaque by bacteria interact with hard structures of teeth in a process in which the composition of enamel determines the susceptibility of these structures to attack. A special role seems to be played by fluoride which favours the formation of apatites having a lesser calcium deficit and more stable crystals.

The aim of our work was to determine the content of some elements in healthy and eroded teeth, i.e. teeth with lesions caused by acids. 14 teeth with enamel erosions were studied in 6 men and 8 women aged 18-30 years. Biopsies were obtained from the labial surface of enamel below (upper dentition) or above (lower dentition) the erosion. The results were compared with healthy teeth of the same location in a group matched for age and sex. An acid biopsy was obtained at the laboratory of the Department of Biochemistry and Chemistry, Pomeranian Academy of Medicine in Szczecin. Fluoride content was determined using gas chromatography (GC-FID). Calcium and magnesium were measured using flame ASA.

Enamel with erosions showed a higher susceptibility to digestion with acid. This was reflected by deeper digestion (1.10 μm) as compared with healthy teeth (0.72 μm). The superficial layer of enamel in eroded teeth contained significantly less magnesium and fluoride (33.49 vs. 55.77 mmol/kg enamel, respectively) as compared with control teeth. Lower content of magnesium and fluoride in the external layer of enamel may reflect changes in the chemical structure of enamel covering eroded teeth and may be one of the factors responsible for increased susceptibility to acids. This may facilitate the formation of erosions and lead to clinical symptoms.

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