BIOCHEMICAL EFFECTS

The biochemistry and physiology of metallic fluoride: action, mechanism, and implications

Fluoride is a well-known G protein activator. Activation of heterotrimeric GTP-binding proteins by fluoride requires trace amounts of Al$^{3+}$ or Be$^{2+}$ ions. AlFx mimics a gamma-phosphate at its transition state in a Galpha protein and is therefore able to inhibit its GTPase activity. AlFx also forms complexes with small GTP-binding proteins in the presence of their GTPase-activating proteins (GAP). As phosphate analogs, AlFx or BeFx affect the activity of a variety of phosphoryl transfer enzymes. Most of these enzymes are fundamentally important in cell signal transduction or energy metabolism. Al$^{3+}$ and F$^{-}$ tend to form stable complexes in aqueous solution. The exact structure and concentration of AlFx depend on the pH and the amount of F$^{-}$ and Al$^{3+}$ in the solution. Humans are exposed to both F and Al. It is possible that Al-F complexes may be formed in vivo, or formed in vitro prior to their intake by humans. Al-F complexes may play physiological or pathological roles in bone biology, fluorosis, neurotoxicity, and oral diseases such as dental caries and periodontal disease. The aim of this review is to discuss the basic chemical, biochemical, and toxicological properties of metallic fluoride, to explore its potential physiological and clinical implications.

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Keywords: Aluminum fluoride complexes; G-protein activation; GTP-binding proteins.

EFFECTS ON TEETH

Fluoride profiles in premolars after different durations of water fluoridation in Ho Chi Minh City, Vietnam

In order to evaluate the uptake of fluoride (F) into tooth structures following water fluoridation in Ho Chi Minh City, F concentrations were measured from the enamel surface through the enamel-dentine junction (EDJ) to dentine close to the pulp in premolars. Calcification had been completed in all the teeth before fluoridation. Samples were obtained from fluoridated areas of the City ([F]: 0.7 ppm) after 3 years (eight samples), 6 years (eight samples) and 8 years (nine samples). Samples were also taken from regions outside the fluoridated water supply of the City in 1998 after 8 years of water fluoridation. An abrasive microsampling method was used to determine the profiles of fluoride and phosphorus concentrations. In enamel surfaces, F concentrations tended to increase with increasing periods of
Abstracts

Fluoridation. In dentine close to the pulp, F concentrations also increased statistically significantly with the increasing duration of fluoridation of water (between 6 and 3 years: P=0.006; between 8 and 3 years: P=0.001; between 8 years and 8 years without F: P=0.0001). It was concluded that F concentrations in enamel and dentine had tended to increase gradually with the duration of water fluoridation in Ho Chi Minh City. The increase was most obvious in dentine near the junction with the pulp.

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Keywords: Dental tissue F distribution; Enamel-dentine junction; Fluoride in teeth; Fluoridation; Ho Chi Min City.

Perception of dental fluorosis among adolescents living in urban areas of Ethiopia

The objective of the study was to assess the perception of dental fluorosis among adolescents residing in two urban areas in Ethiopia: Addis Ababa (low-fluoride drinking water) and Nazreth (high-fluoride drinking water). Students aged 12-15 years, enrolled in two junior secondary schools in Addis Ababa (n = 161) and two similar schools in Nazreth (n = 177) responded to evaluative statements concerning the appearance of anterior maxillary teeth. Color photographs depicting dental fluorosis (TF score 2, 3, 5 and 7) in maxillary front teeth were used as reference during structured interviews. A majority of the students from Addis Ababa and Nazreth were dissatisfied with the appearance of teeth having TF scores 2 and above. More than 66% of the students from both areas confirmed a need for dental consultation concerning fluorotic teeth with TF 2. As compared to their counterparts in Nazreth, children in Addis Ababa felt more embarrassed with TF score 2 and 3 (p < 0.01). TF scores 5 and 7 were found unacceptable both in Addis Ababa and in Nazreth. Students in Addis Ababa, more frequently than their counterparts in Nazreth, believed (erroneously) that dental fluorosis were related to neglect on the part of the child. The present findings confirm that fluorotic teeth constitute a social problem among junior secondary school children residing in low-fluoride as well as in high-fluoride urban areas in Ethiopia and, thereby, corroborate the public concern for safe drinking water.

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Keywords: Addis Ababa; Dental fluorosis perception; Ethiopia; Nazreth; Thylstrup-Fejerskov index.
The prevalence and severity of dental fluorosis among 12-year-old schoolchildren in Jordan

Objectives: To assess dental fluorosis, provide baseline data among Jordanian children and to compare fluorosis in incisor teeth between areas with different fluoride levels.

Design: Cross-sectional study among schoolchildren.

Sample and Methods: A sample of 1878 subjects aged 12 years, 940 boys and 938 girls, were randomly selected from 128 schools in urban (69 schools) and rural (59 schools) areas. The Thylstrup & Fejerskov (TF) index was used to record the dental fluorosis on the labial surfaces of incisors.

Results: A total of 18.5% of the children examined showed dental fluorosis. The difference in fluorosis between males (17%) and females (20%) was not statistically significant (P > 0.05). Children in rural areas had a higher prevalence of fluorosis (31.8%) than their counterparts in urban areas (11.3%), a difference that was statistically significant (P < 0.01).

Conclusions and Recommendations: Findings suggest that there is a need for continued monitoring of mottling and further investigation into the fluoride intake from all sources in Jordan. However, there is a need to change the drinking water supplies in the southern region of Jordan. Active steps should also be taken to suppress emissions of vapours high in fluoride from phosphate plants to within acceptable limits.

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Keywords: Dental fluorosis prevalence; Jordan; Thylstrup-Fejerskov index.

ETHICS

Fluoridation: a violation of medical ethics and human rights

Silicofluorides, widely used in water fluoridation, are unlicensed medicinal substances, administered to large populations without informed consent or supervision by a qualified medical practitioner. Fluoridation fails the test of reliability and specificity, and, lacking toxicity testing of silicofluorides, constitutes unlawful medical research. It is banned in most of Europe; European Union human rights legislation makes it illegal. Silicofluorides have never been submitted to the U.S. FDA for approval as medicines. The ethical validity of fluoridation policy does not stand up to scrutiny relative to the Nuremberg Code and other codes of medical ethics, including the Council of Europe's Biomedical Convention of 1999. The police power of the State has
been used in the United States to override health concerns, with the support of the courts, which have given deference to health authorities.

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Keywords: Ethics of fluoridation; Fluoridation; Silicofluorides.

**FLUORIDE MEASUREMENT**

**Rapid method for the determination of trace fluoride and activation of ion-selective electrode**

A method for activating ISE is proposed that can allow determination of the fluoride concentration at ng/mL level with good precision and accuracy. Fluoride ISE is activated in 0.5 mol/L HClO₄ medium and then fluoride is determined in the same medium. The linear range for the determination of fluoride is between 1.00 x 10⁻²–1.00 x 10⁻⁷ mol/L, and the detection limit of the method is 1.0 ng/mL. The advantage of this method is that it is free from the use of TISAB solution while being, time-saving and labor-saving. A mechanism study of the activation of FISE in HClO₄ medium is explained. The method has been used for the determination of trace fluoride in milk and flour with satisfactory results.

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Keywords: Fluoride determination; Ion selective electrode activation; Perchloric acid activation of ISE; Trace fluoride determination.

**HEALTH/BIOLOGICAL EFFECTS**

**Effects of menopause on bone mineral density in women with endemic fluorosis**

**Purpose:** The effects of menopause on bone mineral density (BMD) in women with endemic fluorosis were investigated.

**Materials and Methods:** Eighty healthy Turkish women who lived in and around the city of Isparta were selected randomly and enrolled in this study. They were separated into four groups: group 1, 20 premenopausal women with regular menstrual cycles and endemic fluorosis; group 2, 20 postmenopausal women with endemic fluorosis; group 3, 20 premenopausal normal women constituting one control group; and group 4, 20 postmenopausal normal women constituting the other control group. Bone mineral density
was measured in the lumbar spine and proximal femur using dual-energy x-ray absorptiometry.

Results: In the premenopausal group, BMD values of vertebrae L2-L4 and Ward's triangle in women with endemic fluorosis were significantly greater than the respective values in women without endemic fluorosis (P = 0.024, P = 0.036). There were no differences between the groups in BMD values of the femoral neck (P = 0.156) and intertrochanteric area (P = 0.076). The BMD values of vertebrae L2-L4, the femoral neck, intertrochanteric area, and Ward's triangle in the postmenopausal women with endemic fluorosis were significantly greater than those of postmenopausal women without endemic fluorosis (P < 0.001, P = 0.015, P = 0.002, and P < 0.001, respectively). The BMD values of vertebrae L2-L4, the femoral neck, intertrochanteric area, and Ward's triangle in the premenopausal women with endemic fluorosis were significantly greater than those of postmenopausal women with endemic fluorosis (P = 0.010, P = 0.002, P = 0.004, and P = 0.010, respectively). The BMD values of the sites noted for the premenopausal controls were significantly greater than those of postmenopausal controls (P < 0.001, P < 0.001, P < 0.001, and P < 0.001, respectively).

Conclusions: Postmenopausal BMD values in both endemic fluorosis and controls were significantly less than premenopausal BMD values. Although the differences were less prominent in women with endemic fluorosis, menopause is still the major determinant of BMD in the spine and femur.

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Keywords: Bone mineral density; Endemic fluorosis and menopause; Fluorosis in Turkey; Menopause; Postmenopausal women.


Respiratory symptoms and lung-function changes with exposure to five substances in aluminium smelters

Objectives: To determine whether exposure to five different occupational substances contributes to respiratory symptoms in aluminium smelter workers.

Methods: A cross-sectional survey of 1,615 male employees of two Australian aluminium smelters was conducted in 1995. Subjects underwent spirometry and were asked about respiratory symptoms and the relationship of those symptoms to work. Their job histories were combined with a task exposure matrix to produce individual quantitative measures of cumulative exposure to fluoride, sulphur dioxide, inspirable dust, the benzene-soluble fraction of coal tar pitch volatiles (BSF), and oil mist.
**Results:** After adjusting for smoking and age, we found that subjects with the highest cumulative exposure to fluoride (>0.16 mg/m³ years) and inspirable dust (>2.9 mg/m³ years) were two to four times more likely to report work-related wheeze and chest tightness than were unexposed subjects. Lower prevalence ratios for the same symptoms were seen with sulphur dioxide and BSF. Levels of lung function decreased slightly with exposure to oil mist, but not with cumulative exposure to other substances.

**Conclusions:** This study suggests that the relevant causative agents for respiratory symptoms in aluminium smelters are fluoride and inspirable dust.

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Keywords: Aluminium smelter workers; Lung-function changes; Occupational fluoride; Respiratory symptoms.


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**Toxic effect of fluoride on reproduction of female mice and development of their offspring**

The toxic effects of fluoride on pregnant mice and their offspring during and after pregnancy were studied. Female adult mice drank a 200 mg/L NaF solution. The mating rate, the pregnancy rate, the changes of body weight of their offspring, and the morphological and functional changes of the thyroid in mice and their offspring were observed. There was no difference in the mating and pregnancy rates (P >0.05); but the body weights of stillborn and newborn mice in two experimental groups seven days after birth were significantly higher than in the control group. (P <0.001). The tetraiodothyronine (T4) concentration in the mice drinking the high fluoride solution during pregnancy and in their offspring was higher than in the control group (P <0.01 and <0.001 respectively) with changes in the morphology consistent with the functional changes. There were some toxic effects of the high concentration of fluoride on the reproduction of female mice and development of fetal and newborn mice. The morphological and functional changes in the thyroid glands of the mice and their offspring induced by fluoride interfered with the development of the latter.

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Keywords: Body weight and NaF; Fluoride and reproductive effects, Female mice, Fluoride toxicity.

Fluoride-induced apoptosis in human epithelial lung cells (A549 cells): role of different G protein-linked signal systems

In the present study, possible mechanisms involved in fluoride-induced apoptosis in a human epithelial lung cell line (A549) were examined. Sodium fluoride (NaF) induced apoptosis in the A549 cells, with a maximum at 5-7.5 mM after 20 hours of exposure. The number of cells with plasma membrane damage (PI-positive cells) increased moderately up to 5 mM, but markedly at 7.5 mM. Deferoxamine (an Al³⁺ chelator) almost completely prevented these NaF-induced responses, which may suggest a role for G protein activation. The apoptotic effect was partially reduced by the PKA inhibitor H89. NaF induced a weak but sustained increase in PKC activity, whereas the PKC activator TPA induced a transient effect. TPA, which enhanced the NaF-induced PKC activity, was not apoptotic when added alone, but facilitated the NaF-induced apoptosis and the increase in PI-positive cells. PKC downregulation induced by TPA pretreatment almost completely prevented the NaF-induced apoptosis and the increase in PI-positive cells. Pretreatment with the PKC inhibitor GF109203X, which abolished the PKC activity after 3 hours, enhanced the NaF-induced apoptosis. KN93 (a CaM kinase II inhibitor) and W7 (a calmodulin inhibitor) seem to reduce the apoptotic effect of NaF, whereas BAPTA-AM (a Ca²⁺ chelator) was without effect. The tyrosine kinase inhibitor genistein also markedly reduced the NaF-induced apoptosis, whereas the PI-3 kinase inhibitor wortmannin augmented the response. In conclusion, the present results suggest that NaF induces an apoptotic effect and an increase in PI-positive A549 cells via similar mechanisms, involving PKC, PKA, tyrosine kinase and Ca²⁺-linked enzymes, whereas PI-3 kinase seems to exert a counteracting effect.

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Keywords: Apoptosis; Deferoxamine; Epithelial lung cells; Fluoride-induced apoptosis; G-protein signaling; Phosphatase kinase activity.

Occupational exposure to fluorinated hydrocarbons during refrigeration repair work

This study describes refrigeration repair workers’ occupational exposures to halogenated refrigerants, focusing on difluorochloromethane (HCFC 22), tetrafluoroethane (HFC 134a) and a mixture of tri-, tetra- and pentafluoroethane (R404A) in 30 work operations. Unlike earlier reported studies, the present study includes working procedures involving welding in order to measure possible occupational exposure to decomposition products. The
measurements included hydrogen fluoride (HF), hydrogen chloride (HCl), phosgene (COCl₂) and volatile organic compounds (VOC). The exposures were assessed during work operations on small-scale cooling installations like refrigerators and freezers. The repair workers’ occupational exposures to refrigerants were moderate, and the major part of the exposures were associated with specific working procedures lasting for relatively short periods of time (<20 min). During these exposure events the concentrations were occasionally high (up to 42434 mg/m³). Although welding operations lasted only for short periods of time, HF was detected in 9 out of 15 samples when HCFC 22, HFC 134a or R404A had been used. Hydrogen chloride was detected in 3 out of 5 samples in air polluted with HCFC 22. Phosgene was not detected. A large number of VOCs in various concentrations were found during welding. Except for the applied refrigerants, halogenated compounds were only found in one sample.

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Keywords: Chlorofluorocarbons; Halogenated refrigerants; Occupational exposure.

HEALTH/TOXIC EFFECTS IN ANIMALS

Effects of chronic fluorosis on electrocardiogram in sheep

This study was carried out to evaluate the effects of chronic fluorosis by means of the electrocardiograms in sheep. Ten sheep with fluorosis living around a volcanic mountain (Tendurek Mount) in East Anatolia in Turkey and 10 healthy sheep were used. Leads I, II, III, aVR, aVL, aVF, V₂, V₄, and V₁₀ were recorded in the electrocardiographs of the sheep. All waves were seen in all derivations. The P-Q interval was significantly (p<0.05) prolonged and sinus bradycardia was observed in the sheep with fluorosis. As a result of this, the number of heart beats was decreased significantly (p<0.05); that is, the number of heart beats was 110 ± 15 in the control group and 75 ± 10 in sheep with fluorosis.

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Keywords: Electrocardiogram in sheep; Chronic fluorosis; Fluorosis in sheep.
Toxic effect of fluoride-arsenic on the reproduction and development of rats

To determine the effects of the fluoride-arsenic exposure on the reproductive function and the development of offspring. Two generations-one nest reproduction was studied in Wistar rats to assess the changes of reproductive function and structure of ovary and development of their offspring after rats were exposed to different doses of fluoride (NaF)-arsenic (As$_2$O$_3$) orally. The rates of the pregnancy, normal parturition, survival and feeding survival in high dose group were lower than that in low dose groups and control. The rates of pregnancy were 73.08% and 61.54% in high dose groups, and 92.31% and 84.62% in the control. But the contents of fluoride and arsenic in rats’ offspring were increased. The weights of rats’ offspring in high dose group were lighter than that in control. The average weights were 38.45 g in high dose group and 60.84 g in control one month after birth. Pathological changes could be found in each stage of oocytes with transmission electron microscope(TEM).

Conclusion: Fluoride-arsenic exposure has adverse effects on the reproductive system and the development of rats’ offspring.

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Keywords: Arsenic-fluoride exposure; Arsenic trioxide; Pregnancy rates; Rat reproduction.