

The effects of fluoride on human health in Eastern Rift Valley, northern Tanzania

Eirik Storøy Johansen



Prosjektoppgave ved Det medisinske fakultet

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<http://www.duo.uio.no/>

Trykk: Reprosentralen, Universitetet i Oslo

Abstract

Exposure to elevated levels of fluoride has effects on human health. In the Eastern Rift Valley obvious clinical effects are seen on the population, dental fluorosis is a widespread problem in the area. The high concentrations of fluoride in the drinking water come from the alkaline volcanic activity associated with the geologic processes of the rift valley. For the majority of the effects on health the mechanism behind remain uncertain. Influence on the intracellular signaling pathways is one of the mechanisms. The scientific literature on the field is contradictory and confusing. Experiments with physiological concentrations of fluoride and better integration between the research disciplines should be done.

Introduction

My motivation for writing about this subject dates back to a trip I made in the winter of 2010 to the eastern side of the African Rift Valley in northern Tanzania. More defined a half an hour north of the city of Arusha, in the south western shadow of the active volcano Mount Meru, in the district of Ngaramtoni. My travel companions were a friend from the medical school and her mother, a medical doctor. My friend had, on a previous trip to Tanzania, become aware of the brown teeth in this area. On that trip she got to know a family, the father a doctor of geology, through the youngest son. The phenomenon of the brown teeth was explained by this knowledgeable head of family. It was due to exposure to fluoride in the drinking water in the childhood years. His family was an example of the effects of fluoride, as his oldest son was born and had his first years in Sweden during the doctoral studies. This son had no sign of dental fluorosis. The subsequent children, born and raised in Arusha, all got the brown lesions of dental fluorosis. Then the first granddaughter of the family was born after the family got a bone charcoal filter that removed the fluoride from the water. She, again, has no signs of dental fluorosis.

My friend was inspired to help and came back with her mother and me one year later. The intention of our trip was to start a project to help the people of Ngaramtoni district preventing the destructive effects of fluoride. The problem and the solution seemed simple enough. With the youngest son, as guide and translator, we talked with local officials and were shown around by representatives from some of the local church communities. We visited 81 families in the villages of Saitabau and Oiloirien, and they were all affected to a different extent by dental fluorosis. We could also see some with signs of skeletal fluorosis. We encountered only two persons born and raised locally with no affection. Of 185 people asked, 84 had musculoskeletal complaints, indicative of the early stages of skeletal fluorosis. This made a strong impression and our intent to help was strong.

The problem and solution seemed straight forward, to make filters filled with bone charcoal and provide them to each household along with information. After that the families would be able to maintain the filters themselves with information and help from a local NGO. The problem and its solution(s) are, however, not so simple.

The questions that arose were many: Who were responsible for people's access to water? Which water sources did people use? What is the prevalence of dental fluorosis? But also: What other effects does fluoride have on the body and, which impact could it have on people's health economy and even happiness? How would the concentrations of fluoride vary and what is the correlation between symptoms and amount of fluoride ingested? How does fluoride actually work once it gets into the body? ...and many more! Some of these questions were easy to find an answer to, others not so easy.

In order to get more information, the scientific literature has been searched. There are many articles and several books describing the occurrence of fluorosis around the world and the different sources of fluoride. And lots of articles that describe the symptoms of the conditions that arise. Some suggest mechanisms for the effects, and others suggest the opposite. There are also a lot of articles on experiments on effects of different cells in vitro, but most of these fail to describe how fluoride exerts its effects.

It shows great holes in knowledge of the effects on cellular level and about the mechanisms behind the clinical conditions that we saw on our trip and of those I have read about later. I was disappointed to discover how poor the communication of ideas is between the laboratories and the societies that they should serve. Community medicine seems satisfied

looking at the mechanisms behind the conditions like a black box and to have research results that only describe epidemiology, while biomedical research is satisfied with experiments that show results, including those done with so extreme fluoride levels that they are irrelevant to the problems seen in the real world.

The results of my literature review show this lack of knowledge and the confusion and complexity of the problem. One of the reasons for this may be the frequent biphasic effects of fluoride, which few articles mention or consider, even the reviews. An example of biphasic effect: is that appropriate, low concentrations of fluoride have a positive, fortifying effect on teeth enamel and prevent caries, but higher values have the opposite effect; dental fluorosis and destruction of the teeth. For other effects, like those that are intracellular, results are often contradictory and confusing. The researchers seem to avoid commenting on different concentrations used in different experiments like this is not a problem when speaking of and comparing effects. This leads to a lot of confusion.

Another reason may be because the authors, in their haste for publication, disregard the imperative to educate the reader, consider connections and put their research into a context. Emphasis on context could not only challenge poorly integrated basic research articles, but also contribute to society implementation and would identify new fields of research and reduce the lack of relevant knowledge.

I wanted to shed light on the problems and questions we encountered in Tanzania in order to come closer to the goal of our project, which was to rid the people of toxic effects of fluoride. To do that, I found that an understanding of the sources of fluoride to man was important. Furthermore, to understand how it gets into the body, the underlying effects and physiological mechanisms in the organism and some of the effects that are clinically seen. The frame of this paper imposes some limitations. I have chosen to investigate some clinical themes; the infamous effects on teeth and bone, the effects on the immune, endocrine and neurologic systems and some of the underlying biochemistry.

Working on this paper has thought me much about scientific literature, about the complexity of community health, geology and some about fluoride's effects on the organism. The paper has three parts. In the results there are two parts: Why is there so much fluoride in this part of Tanzania? The emphasis is on geology, petrology and hydrogeochemistry. The second part asks: What effects does fluoride have in the organism? With focus on some organ systems and basic biochemistry. The final discussion tries to draw some conclusions.

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Method

The searches have been performed in the database for health, PubMed, and for geology, GeoRef database. To get an overview of themes Wikipedia has been a valuable starting block for insight and inspiration for further search. Librarians in the Medical Library and the Science Library have given guidance to books on different subjects.

The books have been found in BIBSYS. Introduction to Medical Geology by Dissanayake and Chandrajith, Mertz: "Trace Elements in Human and Animal Nutrition", McKnight's Physical Geography and Essential Cell Biology.

The search on line started with normative literature, the books, and from the large organizations of WHO and UNICEF. On the home pages of both organizations the search option led to a report on "Fluoride in drinking water" by Chilton et al. (2006). And its references led to other articles found in PubMed, searching for the title.

After the normative literature, reviews were traced to give a general insight in a theme. There after more specific articles from reference lists of review papers or in themes that lacked information on specific parts.

In PubMed the first search "fluoride+drinking water" resulted in 1844 results. To reduce, "rift valley" was added to the search string, resulting in 13. Out of these there was only one study from the specific area of interest, Tanzania. A further interest in the metabolism of fluoride led to the search "fluoride+metabolism" resulting in 18054 results. This seemed too comprehensive and was modified to "fluoride+biochemistry" which led to 1958 results.

Filtering for Reviews reduced the number to 46, and from these one seemed to explain several aspects and was available in full text. In this review there were references to other articles and reviews which were found in PubMed.

In working on the literature and in conversation with the teaching supervisor the curiosity for effects on the immune system arose. The search "fluoride+immune system" led to 1362 results, these were further limited by adding "macrophage" to the search, leading to 243 results. Some of these looked promising of explaining fluoride's effects and were available in English and full text.

A curiosity for the geologic reasons of fluoride in the rift valley arose. This led to the book on Physical Geography, the GeoRef database and an unpublished article from a colleague of the teaching supervisor. In GeoRef the search: "Fluoride +rift valley" resulted in 11 results; one of which was very interesting in respect to my area of special interest, Tanzania.

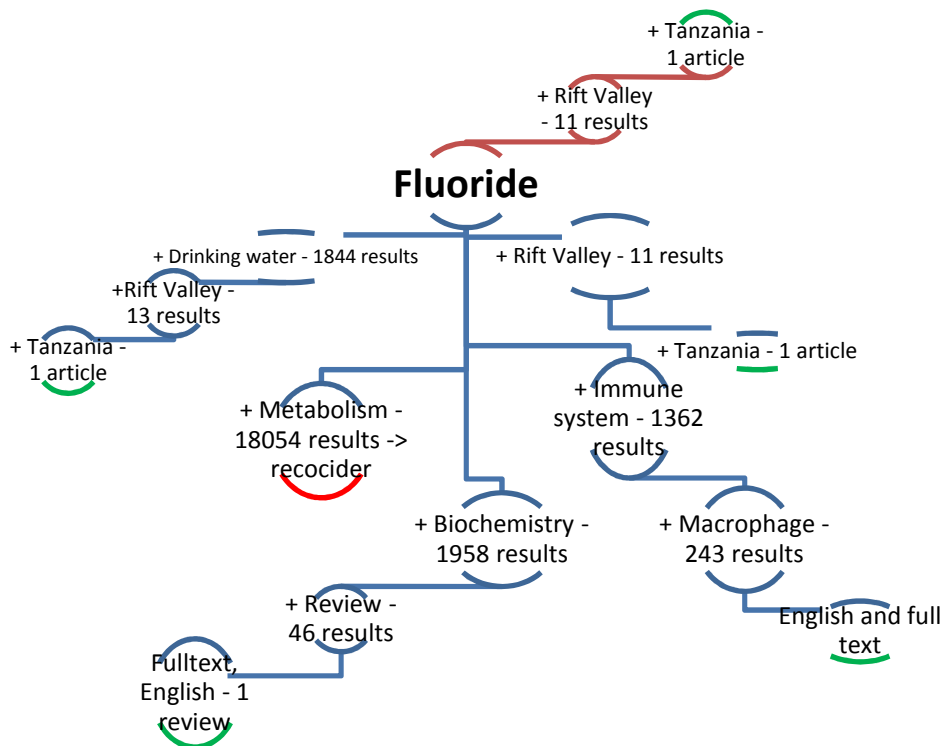


Fig. 1: "Search tree"

Results

Why are there so much fluoride in the Eastern Rift Valley and this part of Tanzania?

The Great Rift Valley system spans from Syria to Mozambique. From Mozambique, north, through Lake Malawi. Here it splits in the Western Rift Valley and the Eastern Rift Valley. The Western turns northwest between Tanzania and the Democratic Republic of Congo, through Lake Tanganyika, north, through Rwanda and the lakes west of Lake Victoria to Uganda. The Eastern Rift Valley goes north, through Tanzania, west of Mt Kilimanjaro, through the highlands of Kenya and Ethiopia to the triple junction in the Afar-region. Here the Eastern Rift Valley meets the rift that is now the Red Sea that goes north to Syria and the rift that is now Bay of Aden.

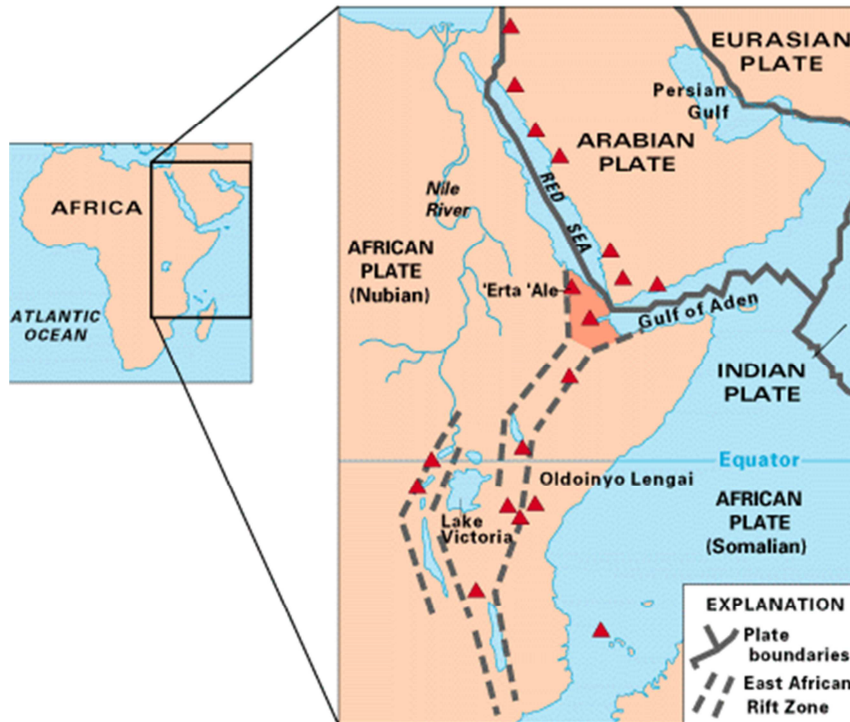


Fig 2: Map of the Great Rift Valley System.

From (<http://upload.wikimedia.org/wikipedia/commons/5/5a/EAfrica.gif>)

Mt Meru and the neighboring Kilimanjaro are volcanoes that are a part of the Eastern Rift Valley System. This is made when the tectonic plate called the African plate is broken, creating the Nubian plate with most of Africa, and the Somali plate. These two are drifting apart, diverging, and are also parting with the Arabian plate to the north. This parting is thought to be because of an increase of heat flow from the mantle of the earth, a mantle plume, causing an expansion and a rise of the crust (See figure 2) (Hess, 2013).

Anatomy of earth:

Crust is the hard outer layer of rock. It is from 7 to 70 km thick.

Mantle is subdivided in 3 parts. The *Lithosphere* lies under the crust and is rigid and goes down to 65 -100 km. The crust and the lithosphere make the plates in “plate tectonic”.

Under the lithosphere comes the *Asthenosphere* extending down to 350 km depth. The asthenosphere is “plastic” - the material is easily deformed, resembles tar and is the source of “lava” and “magma” as we know it on or near the surface. The *Lower mantle* extends down to the core. Here the material is for the most part rigid again due to high pressure.

Core is the source of the heat driving the movement of the above lying magma. It is divided into two parts: *Outer core* is probably molten, stretching from about 2900 km to about 5000 km. *Inner core* is solid and has a radius of about 1450 km. (Hess, 2013)

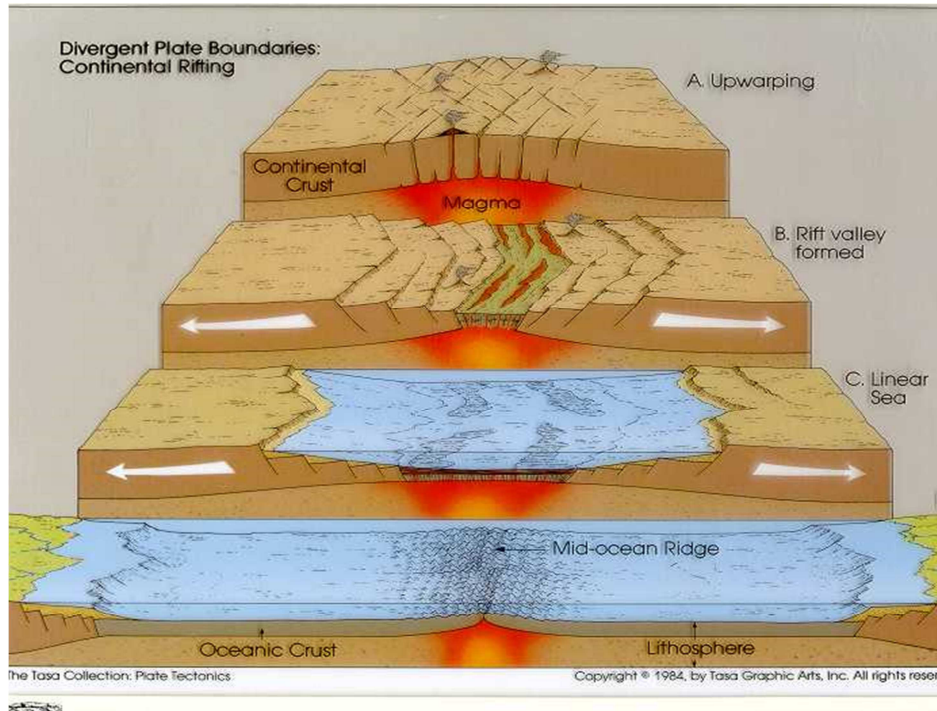


Fig 3: parts A and B showing the intracontinental divergence as of today. Parts C and D showing the possible future, as has happened in what is now the Red Sea. From (http://blue.utb.edu/paullgj/physci1417/Lectures/Plate_Tectonics.html).

This expanding from the heat will make it crack, as seen in highlands in Kenya and in the north and central parts of Ethiopia. This cracking, expansion and divergence of the plates will make faults. In the faults, lava will leak out through fault lines and create volcanoes. It is agreed that the source of fluoride in the northern part of Tanzania stems from the alkaline, volcanic activity in the area.

Volcanic activity

Volcanoes spew out lava, or molten rock. This molten, or igneous, rock comes from deep in the earth, and brings with it fluorine in its minerals (see next section).

There is a lot of volcanic activity associated with the African Rift Valley. Mt Kilimanjaro and Mt Kenya being the tallest extinct volcanoes in the region. Mount Meru is one of the younger extrusive volcanoes with its latest eruption in 1877. (Nanyaro, Aswathanarayana, Mungure, & Lahermo, 1984). The pyroclastics ejected then were probably very fluorine rich, based on measurement from a neighboring and similar volcano Oldoinyo Lengai, west of Mt Meru,

Volcanism

Pyroclastics are material thrown into the air by explosive, volcanic eruptions.

Lahar is a volcanic mudflow. Ash and pyroclastic material sit like a loose mantle on a volcano and easily become mobilized by heavy rain or melting of snow or a glacier.

Rocks are consolidations of minerals. *Igneous rock* is formed by the cooling and solidification of molten rock. Magma is molten rock beneath the surface of the earth and lava is molten rock on the surface. Examples are granite and basalt.

Sedimentary rock is formed by deposition of sediment in a quiet body of water. The accumulated pressure adheres the particles of the sediment to each other or cements them together. Examples are sandstone, shale and limestone.

Metamorphic rocks are originally either igneous or sedimentary, but are physically, by heat and pressure, or chemically altered. The mineral components are recrystallized and rearranged. Examples are gneiss, slate and marble.

(Hess, 2013)

with eruption in 1960.

In this area one finds special conditions due to alkaline volcanic activity. The minerals of the pyroclastics contain unusual high amounts of sodium (Na) and fluorine (F) and low concentrations of calcium (Ca) and magnesium (Mg).

Minerals and chemistry of fluorine

Fluorine forms minerals with properties that, with the right environment, cause dramatic fluorine content in water.

Fluorine is an element of the halogen group. Its atomic number is 9. It is the most electronegative element of the periodic table, giving it its high reactivity, or oxidizing capacity. This is also the reason for its great strength of bonds in the compounds it forms. Due to its high reactivity fluorine is not found free in nature. As a halogen, fluorine needs one electron in its outer shell to become a stable inorganic fluoride ion (F^-). It readily takes the place of hydroxide (OH^-) ions in minerals or compounds, as their ionic radii are similar (Jha, Mishra, Sharma, & Damodaran, 2011).

Fluoride is found mainly in the mineral fluorite, fluorapatite and cryolite. Fluorite is (Jha et al., 2011).

Fluorite CaF_2 , is colorful and found worldwide. Associated with quartz, calcite, barite and dolomite.

Fig. 4: 2,2 cm large, pink crystals

From

<http://en.wikipedia.org/wiki/File:Fluorite-270246.jpg>



Fluorapatite ($Ca_5(PO_4)_3F$)

contains phosphate and is primarily mined for this. The fluorine is discarded.

Fig.5: 1,2 cm large, purple crystals. From

<http://upload.wikimedia.org/wikipedia/commons/b/ba/Apatite-%28CaF%29-89638.jpg>



Cryolite (Na_3AlF_6) is the least common of the three.

Fig. 6: 4 cm large.

From

http://en.wikipedia.org/wiki/File:Iviglut_cryolite_edit.jpg



According to Gerasimovsky and Savinova (1969), apatite is the only F-bearing mineral observed in alkali rocks of the rift zone. Rocks in the rift system, and especially in the northern Tanzanian part of the rift, are distinctly more enriched in fluorine than analogous volcanic rocks elsewhere (sited from Nanyaro et al., 1984. Page 136)

This content of minerals is mirrored in water samples (Table 1). The alkalinity mobilizes fluoride from fluorite with precipitation of calcium carbonate, because the solubility of fluorite increases with concentration of NaHCO_3 (H is hydrogen, C is carbon and O is oxygen).

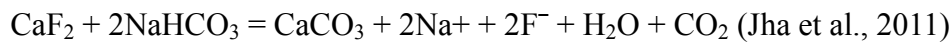


Table 1 showing the concentrations of different ions and pH values of surface water in n. Tanzania.

Table 1. Indicative concentrations of some chemical components of surface and groundwater in the Maji ya Chai and Engare Nanyuki drainage areas and in the Momella Lakes group

Sample description	pH	HCO_3^- (mg/l)	CO_3^{2-} (mg/l)	Cl^- (mg/l)	F^- (mg/l)	Ca^{2+} (mg/l)	Mg^{2+} (mg/l)	Na^+ (mg/l)	K^+ (mg/l)	NO_3^- (mg/l)
<i>Maji ya Chai River system</i>										
Maji ya Chai River by the main road	8.4-8.6	215-340	0-12	17-20	12-14	3-4	<1	150-160	10-30	2-4
Same place during high flood*	7.7	100	0	5	4	2	<1	40	14	8
Spring, Kikatiti	7.9-8.1	960-1230	0	12-15	17-19	4-7	1-2	340-440	120-140	3-5
Pond, Kitefu	8.9-9.0	2500-2750	390-490	160-180	61-65	2-3	1-3	1200-1500	260-270	55-60
Flowing well, Ngongongare	8.2	95	60	15	12	2	1-4	90	27	0
<i>Engare Nanyuki River system</i>										
Engare Nanyuki River near the gate	8.9-9.4	550-700	54-78	70-100	21-26	4-10	1-3	340-450	80-130	4-14
Same place after heavy rains*	9.0	440	63	120	21	4	<1	280	60	9
Jekukumia stream	9.0-9.1	1700-1730	380-400	280-290	58-76	2-5	1-3	1100-1300	270-290	2-8
Jekukumia springs	7.8-7.9	1760	410	310	63	2-5	1-3	1100-1300	270-300	7
<i>Momella Lakes group</i>										
Lake Senato	7.5	n.a.	n.a.	13	<1	15	3	47	21	n.a.
Lake Longil	8.1-8.3	210-250	0-9	11-16	2-4	5	3	72-82	34-38	0
Lake Kusare	8.9-9.3	1320-2100	220-270	75-110	56-65	2-3	1-3	840-910	110	9
Lake Kekhotoito	9.1-9.4	2000-2400	200	110-130	75-86	3-6	1-2	1100-1300	140-170	14
Small Momella Lake	9.2-9.8	2050	790	120-180	110	<1	2	1200-1400	190-210	(9)
Same place after heavy rains*	9.4	1700	500	200	110	1	2	1400	200	30
Lake Reshitani	9.7-10.0	3550-4070	3660-5840	430-680	300-450	1-4	1-3	4100-4200	590-670	30
Big Momella Lake	9.6-10.0	3750-4000	4000-6400	490-750	380-560	1-4	4-5	4800-6000	700-980	28
Lake Tulusia	9.7-9.8	4100-4300	4700-7200	650-950	440-690	1-3	4-6	4600-6600	730-850	30

The ranges of concentrations were calculated from one to three samples collected in May-June and September-October, 1981-1982. The samples are from a comparatively dry season except for those marked *, which were collected after exceptionally heavy rains causing flashfloods by lowland rivers. The one set of analysis for the lakes presented (except for Lake Senato) was collected from Kilham (1971). Heavy rains have a great impact on the chemical composition of rivers, e.g. the Maji ya Chai flowing in lowlands. The dilution effect is slighter in mountain rivers (e.g. the Engare Nanyuki) where there is higher annual rainfall and lower temperature (in this exceptional case also higher TDS contents). No effect is discernible in brackish/saline lakes (e.g. Small Momella Lake). Lake waters are presented in order of increasing F and Cl contents. An increase in F contents is accompanied by an increase in pH, alkalinity (HCO_3^- , CO_3^{2-}), Na and K, while very low Ca and Mg contents make high F concentrations possible.

Examples of water content in the Rift Area of Northern Tanzania. Modified from Nanyaro et al. 1984. Page 132.

These chemical aspects increase the fluorides ability to solve in water.

According to Skjelkvale (1994) aluminum (Al) will form strong complexes with fluoride and reduce the levels of free fluoride in the solution in natural water of lower pH. At increasing levels of alkalinity aluminum forms complexes with OH⁻ groups, allowing high fluoride in solution, reference in Jha et al., (2011)

The ash from an eruption will spread over a large area. Lahars form from the pyroclastic material. The ash and lahars are easily decomposed, and the contents will leach into the surface water, soil and groundwater. The solidified rock takes a much longer time to weather and its minerals to enter the environment (Jha et al., 2011).



Fig. 7: Lahar from Mt. St Helens

From <http://www.mshslc.org/return-to-life/facts-and-research/faqs-on-return-to-life/how-did-the-rate-of-vegetation-re-establishment-compare-on-lahar-mudflow-deposits/>

Drinking water

The contents of fluoride in the drinking water vary a lot, even within the same area, depending on several factors.

People have different sources of drinking water; rainwater, surface water of lakes and rivers or streams and groundwater. In respect of fluoride content, rainwater and surface water may absorb dust or pollutants containing fluoride from coal burning or industry such as oil refining, steel production, chemical production, clay production, aluminum smelting, brick and ceramic manufacture and nuclear industry (Jha et al., 2011).

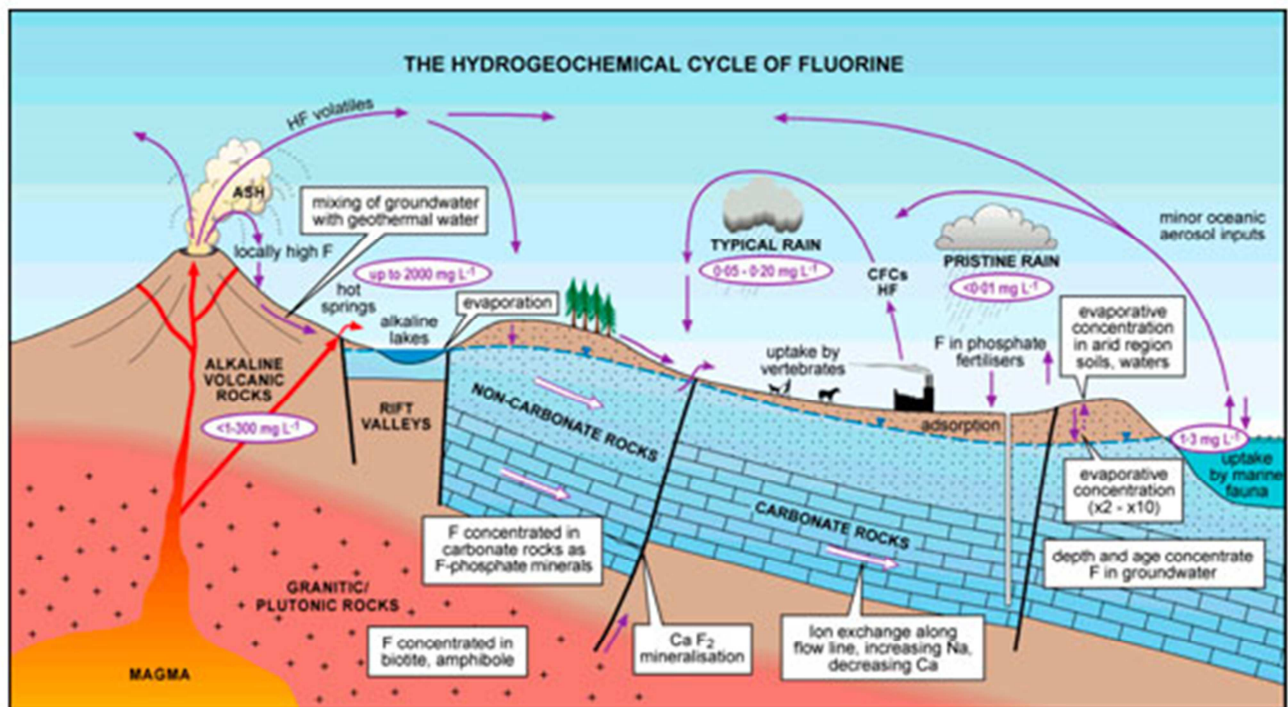


Fig. 8 shows the main sources and sinks of fluoride.
 From <http://www.gwadi.org/tools/tracers/fluoride>

Surface water's content of fluoride varies a lot (Table 1). Variation depends on the source or pollution and on the weather. For example a spring from a groundwater source with high fluoride concentration will contain a lot, and even contaminate rivers or lakes. And after heavy rainfall there will be a dilution of fluoride (Nanyaro et al., 1984).

Evaporation is another factor of great importance. An illustrative example: The alkaline, closed Momella Lakes have a content of around 60-600 mg/l of fluoride, (and they taste like brackish water). The theory of the extreme content in these lakes is from Nanyaro et al. (1984), there will be a continuous inflow of fluoride from seeping groundwater, sporadic over-land flow (dependent on rainfall) and an occasional heavy load from ash fall. And then there is evaporation, concentrating fluoride as water leaves the water body through the air, until saturation with respect to villiamite (NaF). This is allowed as the water, and the area contains such low levels of Mg and Ca, which would have removed fluoride from solution earlier. Water temperature is a factor too. The difference in solubility of fluorite between 10 and 25 °C is markedly higher (Jha et al., 2011). This is an extreme example, but illustrates several effects. Similarity is found at Lake Magadi in Kenya, source of the name of a popular food salt from the area.

The release of fluoride from minerals to the groundwater are determined by “*the chemical composition of the water, the presence and accessibility of fluoride minerals to water and the contact time between the source mineral and water*” (Jha et al., 2011 found in Keller (1979)). In the Eastern Rift Valley area all of these determinants play into effect and increases the levels fluoride in the water. As before mentioned, the area has unusually low concentrations of Ca and Mg witch would inhibit solution of fluoride, the pH of the water is alkaline and will enhance the solubility of fluoride (Table 1). The presence of fluoride is described earlier and the accessibility depends on particle size and types of minerals.

The contact time is also long in this area as it is arable land. In areas with high rainfall, there will be a high turnover of the water in the ground, and shorter time for the water to absorb

ions from the minerals. In arable areas as here, there is limited refill of water from rain (Jha et al., 2011). In general the concentration of fluoride increase the deeper into the ground you come, but it also shows great variability within one area. This increase can be explained by accumulation, on the waters way down from the soil, fluoride is leached and accumulated (Jha et al., 2011).

Lacustrine deposits are made when water reaches a lake, and lets go of the material carried by the moving water, making sediment and eventually rock. (Hess, 2013)

Another contribution to fluorine in ground water is from gas and steam ascending from the magma chamber towards the surface. On its way, it may get dissolved in the groundwater. This may be one reason for high variability of the fluoride concentrations in different ground water pockets (Nanyaro et al., 1984).

The groundwater can be accessed from natural springs or wells. Fluoride contents in the water from the wells depend on depth and local variation. High fluoride containing water came from lahars and lacustrine deposits and comparatively low-fluoride containing water from basaltic or phonolitic unaltered lava at high altitudes (Ghiglieri, Balia, Oggiano, & Pittalis, 2010). Drinking water is probably the most important source of fluoride to man, at least in this area. Other sources are discussed in the next section.

Sources of fluoride to man

According to Jha et al. (2011) groundwater is the major source of fluoride for most people. Though there are other sources such as tea, air pollution, food and manmade products. Main focus has been put on groundwater as this is probably the main source of fluoride in Tanzania. The reason of this is that the temperatures are high, leading to perspiration and increased need of water as compared to a temperate climate. With levels of fluoride over 1,5 mg/l and increased need for water, one quickly exceeds the daily dose fluoride that entails increased risk of toxicity.

Fluoride from food is a limited source because of fluoride's bioavailability. Fluoride is not taken up through active transport in plants roots and therefore the concentrations in plants remains low, or lower than that in soil (personal communication, O. A. Christophersen). The ratio between ash from plants and soil was given 0,2 to 0,6 by Bieliyakova (1977) as referred to by Jha et al. (2011).

But, in the northern Tanzania area, importance is laid on use of the “trona” or “magadi” salt for flavor in cooking. Magadi salt gets its name from the Lake Magadi area and contains high concentrations of fluoride. In families that use this salt there is seen more pronounced dental fluorosis. Tea can also contain high concentrations and be of significant contribution (Jarvis, Heslop, Kisima, Gray, Ndossi, Maguire, & Walker, (2013).).

In areas that are dependent on coal burning for warmth and cooking, fluorine in the inhaled smoke is one contributing factor. Also in industry workers this is a major factor for fluoride exposure. This is a greater problem in India and China (Dissanayake & Chandrajith, 2009). Dental products and treatment contain very high levels of fluoride. This will to some extent be internalized and so a source of fluoride to the individual. It will also contribute to the environment and water from the industry and in wastewater.

Whatever the source, the organism is exposed to different concentrations of fluoride. As in most cases of exposure to living organisms, fluoride's effect on the recipient is dose and duration dependent.

What effects does fluoride have on the organism?

There are many health problems in man claimed to be caused by fluoride. Some are: reduced immunity, deformities of red blood cells, effects on hormones, repeated abortion or still births,

neurological manifestations, malaise, depression, gastrointestinal problems, urinary tract malfunction, muscle fiber degradation, male sterility, reduced intelligence as well as the most famous dental and skeletal fluorosis. Only some will be discussed here, dental and skeletal fluorosis, neurological manifestations and effects on the immune and endocrine systems. From the acquisition of fluoride, throughout the body's organs and cells, fluoride has many functions and effects.

Metabolism of fluoride in humans

The body's absorption of fluoride depends largely on the diet and nutrition. Some of aluminum's metabolism is also discussed here, as their presence in the body is proposed to be linked.

Intake of fluoride with food retards absorption and reduces bioavailability from near 100% with fasting to 70% with a glass of milk, and to 60% on a calcium-rich meal according to Ekstrand and Ehrnebo (1979) and Shulman and Vallejo (1990) referred to by Jha et al., (2011). This reduction is probably from binding to Ca or other divalent or trivalent cations in the food and will probably result in increased fecal excretion of fluoride (Jha et al., 2011). Krishnamachari (1987) reports that this increased fecal excretion with Ca ingestion is not very high, and that it did not make a difference to the plasma levels of fluoride. Li (2003) sees it as more likely that AlF_3 is absorbed from the gut than any other complex, due to the chemical conditions in the gut and properties of Al and F. Al on its own is hardly absorbed from the gut, just 0,1% of dietary intake, resulting in a serum concentration of 6-7 $\mu g/l$.

The levels of fluoride in question are as follows: To prevent caries the recommended levels in the drinking water are 1-1,5 mg/l, as one sees increasing caries at levels of fluoride under 0,5 mg/l. This is for temperate climates, and should be lower for drier climates (Chilton et al., 2006). 3-6 mg/l is considered to cause risk of moderate adverse effects, such as severe dental fluorosis. 6-10 mg/l causing severe adverse effects such as skeletal fluorosis. Over ten and onwards gives increasing risk of crippling skeletal fluorosis, as discussed below (Nanyaro et al., 1984). The government of Tanzania has been forced to set the limit at 8 mg/l because of overhanging risk of water shortage if the limit is set lower (Ghiglieri et al., 2010).

Once in circulation 99% of the fluoride will be retained in the hard, Ca-rich areas such as bone and enamel (Chilton, Dahi, Lennon, & Jackson, 2006). Al in the body accumulates on bone surface, and is buried in the bone matrix as the bone grows (Li, 2003).

The concentration of fluoride in the tissues is a reflection of the concentration in the blood. Plasma concentrations of fluoride in human varies from 13,3-45,6 $\mu g/l$ (Li, 2003). Of the ingested fluoride, about 50% is incorporated in the hard tissues within 24 h, the rest is excreted through the kidneys (Dhar & Bhatnagar, 2009).

In the tubules of the kidneys there is a concentrating process and they see higher levels than the plasma. According to Gedalia (1970) (sited by Jha et al. (2011)) the placenta regulates the transfer of fluoride to the fetus. Krishnamachari (1987) describes both situations in experimental animals, that fluoride crosses the placental barrier freely, and that it does not cross at all. On further investigation it is concluded that fluoride in fetuses matches that in the mother. Though it is lower towards the end of the pregnancy, perhaps as a sign that the bones of the fetus start absorbing fluoride. This illustrates the confusion in the literature.

According to Ekstrand et al. (1984), fluoride is poorly transported from plasma to milk. The content of fluoride in the saliva mirrors that in plasma unless topically applied (sited by Jha et al. (2011)). This is of importance to the effects of the oral cavity, which will be investigated in the next section.

Effects of fluoride seen on teeth

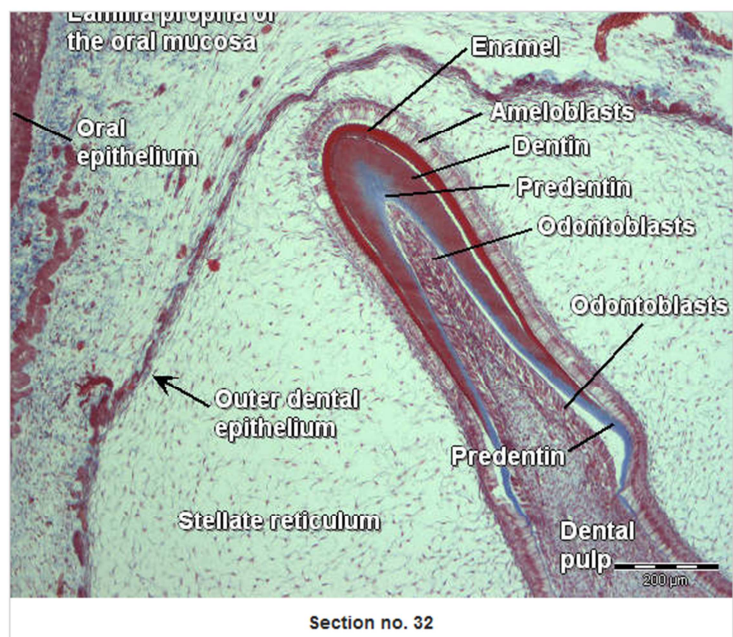
A good example of dose dependent effect is seen in the oral cavity, from preventing disease to causing great damage. The effects are ranging from mineral chemistry to biochemistry of enzymes in the host and in the bacteria. Well established is the effect on protection from dental caries. This effect is the rationale behind the government policies all around the world of adding fluoride to the drinking water, to a concentration of about 1 mg/l, which is considered a healthy concentration (Chilton et al., 2006). There are several mechanisms underlying this effect.

It is well established that fluoride reduces acid production by inhibiting the carbohydrate metabolism of the acidogenic plaque flora found by Hamilton (1990) according to Li (2003). Further it has been found that aluminumfluoride complexes (AlF_x) (more on these in later segments) inhibit an adenosine triphosphate (ATP)-proton pump in the walls of bacteria. This causes hydrogen ions to build up inside and acidify the interior of the bacterium (Li, 2003). Fluoride also probably inhibits bacterial peroxidase-enzymes in the acidic bacteria plaques, and so inhibiting the bacteria's ability to cause caries. This inhibition stopped when the pH rose, and so not inhibiting the peroxidases of the saliva (Thibodeau, Bowen, & Marquis, 1985).

Enamel formation or amelogenesis consists of two stages. In the secretory stage ameloblasts lying on top of the dentin bud will start producing enamel matrix consisting of different proteins and minerals and excreting this between themselves and the dentin. In the maturation stage some of the proteins are broken down by enzymes secreted by the ameloblasts and reabsorbed by the ameloblasts and increasing mineralization of the matrix and organization of the enamel rods take place (Aoba & Fejerskov, 2002).

Fig. 9

Histologic picture showing odontoblasts producing dentin from below and ameloblasts producing enamel from the top, and the cell groups diverging with increased production.



When fluoride is incorporated into the enamel matrix at “healthy” concentrations, the enamel gets harder and more resilient to chemical breakdown, i.e. less soluble.

The enamel is affected in two ways: By interfering with the cell activities, and by affecting the matrix. First, the effect on the ameloblasts, the cells that make the enamel: They are probably affected by AlF_x (x signifies different numbers of fluoride such as 3 and 4) in the production and secretion of proteinases to the enamel matrix (Li, 2003), although it is

disputed if this is the case in concentrations that humans experience (Aoba & Fejerskov, 2002).

Fluoride probably has an inhibitory effect on the enzymes that brake down these proteins through modulating the Ca-environment and so influencing the Ca-dependent enzymes. And fluoride in the apatite crystals may increase the adsorption of, or “stickiness” of the proteins to the crystals, causing them to be unavailable for degradation. In the end the fluoride damaged tooth enamel contains too much protein in the matrix. This makes the enamel porous.

An example of the biphasic effect is that incorporated into the crystalline structure of the enamel apatite, fluoride makes the apatite less soluble and more resilient to acid. Too much fluoride in the crystalline matrix, however, makes larger gaps between enamel rods and larger intercrystalline spaces and the result is weaker enamel. This occurs in a linear relationship with the higher doses of fluoride. This linear relationship is after the “healthy concentration” has been crossed, and is negative for dental health. A biphasic effect.

After eruption of the tooth, the affected areas may be stained dark, brown or yellow. The porosity caused increases with severity and is seen deeper in the tooth and extensive mechanical breakdown, structural destruction, may follow (Aoba & Fejerskov, 2002).

Dental fluorosis (Fig. 10) will only arise when the individual is exposed to elevated levels of



Example of dental fluorosis. Private foto.

fluoride during the enamel formation i.e. before the tooth erupts. It is considered that the accumulated exposure, not the timing of the exposure, is of highest importance of the outcome. But, there is a correlation to timing, and the most damaging effect is seen when exposure happens at age 1-3 (Aoba & Fejerskov, 2002).

There are different grading systems of dental fluorosis. The Dean index bases its classification on the clinical appearance of the enamel, and it varies from normal to severe:

Table 2

Normal:	Translucent, smooth enamel with a glossy appearance.
Questionable:	Seen in endemic areas, borderline between normal and very mild.
Very mild:	Small opaque, paper-white areas scattered irregularly over the labial and buccal surface of teeth.

Moderate:	Entire tooth surface involved, minute pitting often present on labial and buccal surfaces, brown surface, brown stains, frequently disfiguring.
Moderately severe:	Entire tooth surface involved, marked pitting with intense brown stain.
Severe:	Widespread, deep brown or black areas, corrosion type of mottled enamel.

Thylstrup and Fejerskov (1978) use a system of 10 scores based on microscopic and macroscopic appearance (Thylstrup & Fejerskov, 1978). Besides than teeth, bones are also significantly affected.

Effects of fluoride seen on the skeleton

There are seen effects on the cells forming the skeleton and, to some extent, the mineral structure in bone. As in teeth, one supposes a beneficial and harming effect according to dose of fluoride. The effects on the bones are maybe of greater importance to ill health than that seen in the oral cavity.

Bone mineralization with fluoride present will make fluorapatite instead of hydroxyapatite, fluoride ion taking the place of a OH⁻ in the structure. Fluorapatite has greater crystallinity, larger crystals and lower solubility in water than hydroxyapatite. With fluoride present in preformed apatite, it will reduce its solubility as well, probably by forming a layer by the less soluble fluorapatite on the surface of the apatite (Pak, Zerwekh, & Antich, 1995).

It is seen that fluoride has effect on the skeleton. It causes increased bone mineralization and has been used in treatment of osteoporosis. Li (2003) reports that Farley et al. (1983) found that fluoride increases proliferation and alkaline phosphatase activity in avian osteoblasts in culture.

The problem has been to find the dose that allows the benefits of fluoride, and evade the side effects, as too high levels of fluoride can have an inhibitory effect on mineralization and reduce the mechanical quality of bone. This is probably more from fluoride's influence on the orderliness of mineral deposition in organic matrix, than from the properties of fluorapatite as such (Pak et al., 1995). The amount of fluoride retention in bones depend on the individuals stage of skeletal development. More is retained in young bones than in grown up bones (Dhar & Bhatnagar, 2009).

With too much fluoride one may get skeletal fluorosis, with osteosclerotic and osteoporotic areas and even osteomalacia. Fluoride causes increased bone mineralization, but this at a prize of causing more brittle bone. And, as in severe skeletal fluorosis, added bone mass outside the normal structure, deformation and crippling of the person.

Krishnamachari, 1987 classifies skeletal fluorosis into phases: preclinical, musculoskeletal, degenerative and destructive, crippling and complications. The clinical symptoms of skeletal fluorosis are at first similar to arthritis or spondylitis, arthralgia, malaise, weakness and joint stiffness, sporadic pain, back stiffness, burning like sensation, pricking or tingling in the limbs, chronic fatigue and abnormal Ca deposits in bones and ligaments. At an advanced state

Osteosclerosis: abnormal hardening of bone (like ivory)

Osteoporosis: abnormal loss of bony tissue resulting in fragile porous bones

Osteomalacia: abnormal softening of bones caused by deficiencies of phosphorus or calcium or vitamin D

From:
<http://wordnetweb.princeton.edu/perl/webwn?s=>

bow legs and knock-knees (genu valgum), stiffness of the trunk due to fused vertebrae, impeded movements of the limbs and severe joint contractions (crippling fluorosis) is seen (Jha et al., 2011; Nanyaro et al., 1984). Severe skeletal fluorosis is usually chronic high intake of fluoride combined with malnutrition, strenuous manual labor and impaired renal function. This effect is of course not limited to man, but is also seen in livestock and animals drinking water with high levels of fluoride (Krishnamachari 1987).

According to Li (2003) Imai et al. (1996) found that fluoride increases transport of phosphate across cell membranes, and Al potentiate this. Caverzasio et al. (1996) found, according to Li (2003), that fluoride alone had no effect on proliferation, but with Al, it did. Li (2003) also concludes that it is very likely that AlF_x is the active ingredient of stimulation of bone cell proliferation.

“It seems that both F and Al are concentrated in a surface layer of mineral, where active bone growth and remodeling occur (Smith 1985, Priest 1993). It is reasonable to suspect that high concentrations of labile F and Al and their complexes may exist in the extracellular fluid surrounding these cells. This unique microenvironment is the likely site where AlF_x exert their effects on bone cells.” (Li, 2003, page 108)

The proliferative effect on osteoblasts may be due to several mechanisms: First, by manipulation of the enzymes that produce or modulate the proteins that go into the cellular messenger pathway that stimulates mitosis, the mitogen-activated protein kinase (MAP-kinase) pathway. Secondly, by increasing inositol triphosphate (IP_3) in the cytosol and so causing release of Ca from the endoplasmic reticulum (ER). This causes phosphorylation of proteins and possibly synthesis of growth factors. Thirdly, by modulating the effect of existing growth factors on the osteoblasts such as insulin-like growth factor 1 (IGF-1), calcitonin and parathyroid hormone (PTH) (Pak et al., 1995).

That is a short review of some intracellular pathways that fluoride affects. We will now look at some of the effects on the endocrine and neurologic systems.

Effects of fluoride on the endocrine and neurologic systems

The effects of fluoride are, as mentioned earlier, plentiful and sometimes opposing. The effect on Ca metabolism was professed and found, whereas on iodine metabolism not found. The neurologic effects are also, perhaps, of major importance to ill-health in man.

Parathyroid gland increases its metabolism. Fluoride stimulates osteoblasts while calcitonin inhibits osteoclasts; decreased levels of calcitonin have been seen in patients with fluorosis. One sees elevated levels of alkaline phosphatase activity in fluorosis and it also causes excess levels of PTH, hyperparathyroidism. This may be because of interfering with the calcium balance (Koroglu, Ersoy, Koroglu, Balkarli, Ersoy, Varol, & Tamer, 2011) Fluoride toxic influence on thyroid function has been ruled out (Krishnamachari 1987).

Fluoride has inhibitory and stimulatory effects on many enzymes in plants, animals and humans. Some are inhibited in vitro, and enhanced in vivo according to Miller et al. reported by Krishnamachari.

Al is generally considered a neurotoxin and is strongly associated with Alzheimer's disease (AD). One study performed by Still and Kelly (1980), referred to by Li (2003), compared, with respect to hospital-admittance of AD, one county with a high content of fluoride in the water with two counties with low contents of fluoride. They found that the incidence was only one fifth in the high-fluoride county, so fluoride may have a protective effect against AD. Maybe through the effect of G protein and the subsequent pathways (Li, 2003).

On the other hand Varner, Jensen, Horvath, & Isaacson, (1998) reports that administration of low levels of AIF₃ causes damage to the brain and vessels. They also found that slightly elevated levels of NaF do the same, but to a lesser extent.

Choi, Sun, Zhang, & Grandjean (2012), a systematic review on the neurotoxicity and development in children, concluded with significance that children in high fluoride areas had lower IQ-scores than control children. The review criticizes the quality of the reviewed research Krishnamachari (1987) refers to works done by Jolly et al. and Rao et al. describing demyelination and re-myelination in people with skeletal fluorosis. They ruled out primary muscle damage.

Other research using AIF_x added to the food of animals have shown other toxic effects on brain tissue and other tissues (Li, 2003). Some of these tissues are the cells and organs of the immune system.

Effects of fluoride on the immune system

There are indications that fluoride has immunological effects. Effects on cell migration, phagocytosis, inflammatory response and even on the structure of immune system organs.

One research group showed that it was likely that chronic exposure to fluoride causes immunotoxicity by induction of oxidative stress (Das, Maiti, & Ghosh, 2006). They found significantly lower numbers of immune cells; lymphocytes, monocytes and neutrophils, in blood, and reduced levels of immunoglobulin G (IgG) responding to an antigen. Both the spleen and thymus showed disorganization of the histoarchitecture and it was concluded with that the reason for this was oxidative stress.

Another study found that even in small concentrations (1, 3, 6 and 10 μ M) fluorides changes the amounts and activity of 15 lipoxygenase (LOX)-1 and -2 enzymes in macrophages taking part in the development of inflammatory process (Gutowska, Baranowska-Bosiacka, Safranow, et al., 2012).

In macrophages cyclic adenosine monophosphate (cAMP) is important for regulating, among other activities, phagocytosis and migration. However, high intracellular levels are negatively correlated with these two activities. Production of cAMP is described in the fact box, and the mechanism for increased production is found in the next section. These effects were investigated with respect to atherosclerosis, and whether the μ -molar fluoride levels seen in the serum of people who are chronically exposed to high fluoride, would be of importance. At concentrations 4 times higher than the maximum in physiologic levels they

Adenylyl cyclase is and membrane bound enzyme that is activated by the $G\alpha$ subunit of G protein. Adenylyl cyclase will produce cAMP from ATP.

cAMP is a small messenger or second messenger in cells. It in turn activates enzymes in the cell, e.g. protein kinase which in turn activates proteins or other enzymes. This sort of action is called a cascade. The cAMP signal is terminated by cAMP phosphodiesterase turning cAMP into adenosine monophosphate.

Phospholipase C is another target of the G proteins. It cleaves an inositol phospholipid in the membrane. The cleave generates two small signaling molecules. Inositol 1,4,5-triphosphate (**IP₃**) and diacylglycerol (DAG). IP₃ diffuses into the cytosol, binds to Ca^{2+} channels in the ER causing a release of Ca^{2+} ions to the cytosol. **DAG** recruits protein kinase C to the membrane from the cytosol.

Protein kinase C is an enzyme that phosphorylates intracellular proteins and in that way regulate intracellular. It is dependent on Ca^{2+} and DAG to become active.

Ca²⁺, calcium ion, is such an important small messenger in the cell. Its release is governed by other factors as well as those discussed here. The intracellular concentration is normally extremely low, until stimulation. It interacts with Ca^{2+} responsive proteins e.g. calmodulin. Calmodulin undergoes structural change when Ca^{2+} is present, and in this changed form activates Ca^{2+} /calmodulin-dependent protein kinases (CaM-kinase)

Phospholipase A₂ (PLA₂) will be activated by increased levels of cAMP, and make eicosanoids, which are pro-inflammatory molecules from free arachidonic acid

found stimulatory effect on the PLA₂ through several suggested effects (Gutowska, Baranowska-Bosiacka, Siennicka, et al., 2012).

One group found that physiological levels of fluoride (1-4 μM) increased neutrophils ability for phagocytosis of bacteria associated with periodontitis. Two effects were suggested: a stimulation of the cells ability to phagocytize, and increasing the bacteria's susceptibility to being phagocytized (Gutiérrez, Liébana, Ruiz, Castillo, & Gómez, 1994).

Fluoride was found to inhibit PKC (see fact box) in macrophages in an experiment investigating fluoride as a pharmacological tool, using extreme concentrations of fluoride (Hauschildt, Hirt, & Bessler, 1988).

One study indicates that fluoride inhalation inhibits bactericidal activity in the airways, leading to decreased pulmonary antibacterial defense mechanisms. This might play a role in the inability to cope with bacterial infections in individuals residing in fluorosis areas in China (Yamamoto, Katagiri, Ando, & Chen, 2001).

In the next part we will review what is probably one of fluoride's most important mechanisms of affection on the cells of the body, its effect on phosphoryl transfer enzymes and G proteins.

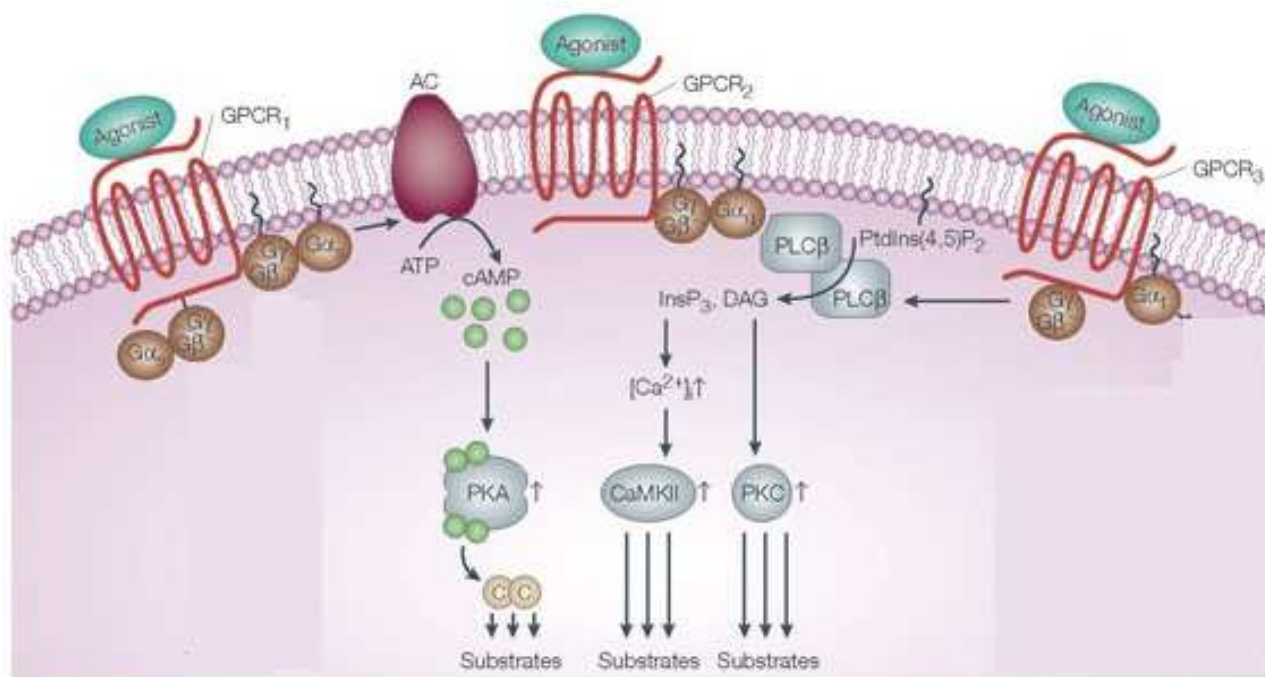
Effects of fluoride on the signaling pathways in cells

In the review by Li (2003) he discuss the effects of metallic fluoride, fluoride in combination with Al or Beryllium (Be) which he concludes is the most probable active form of fluoride in the body. AlF_x and BeF_x are small inorganic molecules that mimic the chemical structure of phosphate, according to Bigay et al., (1987) (sited by Li, 2003), and so will influence the activity of phosphoryl transfer enzymes such as guanosine triphosphatease (GTPase), adenosine triphosphatease (ATPase), phosphohydrolysase and phospholipase D. Phosphoryl transfer is an underlying mechanism of energy metabolism and signal transduction in cells. AlF_x is probably formed both in vitro and in vivo as both elements are common in food and/or drinking water. And several studies in the review by Li indicates that AlF_x, and BeF_x to much less extent, are active in many health aspects.

G proteins are mediators of signaling. They are coupled to a transmembrane receptor. When this receptor receives its signal, it will activate the G protein. G proteins are subdivided in three subunits, G_α, G_β and G_γ. There are different types of G proteins, G_s – stimulates, G_i – inhibits its target, adenylyl cyclase. When G-proteins are activated, the alpha-subunit disengages and activates a target. Adenylyl cyclase, which makes cAMP is one target. This will continue to produce cAMP until the alpha-unit is inactivated and returns to the other subunits of the G-protein.

This inactivation happens from an intrinsic GTPase activity in the alpha-subunit. This will remove one phosphate group from the GTP, making a guanosine diphosphate (GDP). On activation of the alpha-subunit it will substitute a GDP with a GTP. As long as this is in place, as a GTP, the subunit is activated. cAMP is a secondary intracellular, signaling molecule, with effects on the cytosolic enzymes and other signaling pathways in the cell causing a multitude of actions in the cell.

(Alberts et al. 2009)



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Fig. 11: Some G protein signaling pathways.

Modified from http://www.nature.com/nrm/journal/v6/n9/fig_tab/nrm1716_F1.html

Li found that in the end of the 1980s researchers, Bigay et al. 1985 and 1987, Higashijima et al. 1987 and 1991, found that

AlF_4^- interacts with G_α protein through binding to the β -phosphate of GDP. This bound AlF_4^- (or BeF_3^-) simulates the presence of the bound γ -phosphate of GTP and therefore confers on the protein the structure or the active $G_\alpha \cdot \text{GTP}$ state. The high electro-negativity of F^- allows F^- to form strong hydrogen bonds with nearby amino acid side-chains. This tight bonding makes AlF_4^- and BeF_3^- non-hydrolysable by the GTPase activity of G_α , and thus maintains the G protein in its activated state (Li, 2003. Page 102).

This model, of how metallic fluoride affects enzymes, was adopted onto other phosphoryl transfer enzymes.

The stimulation from G proteins may also lead to activation of a MAP-kinase pathway; which is a mitogenic signal transduction pathway leading to stimulation of cell proliferation. AlF_x may also cause disturbances of protein trafficking through the rough ER (rER) and Golgi (Aoba & Fejerskov, 2002).

A general mechanism of affecting cells is fluoride combined with aluminum in a complex, AlF_x , the numbers of fluoride vary between 2 and 3. The AlF_4^- has the same stereochemical configuration as a phosphate group, a tetrahedron. This similarity causes the effect as the aluminumfluoride complex will take the place of the phosphate group in some phosphatase reactions. (Fig. 12)

Best described is the effect on the G-proteins. On inactivation of the G_{α} subunit, in the transition phase, AlF_3 can take the place of the phosphate group that is about to be ejected from the GTP. This switch will inactivate the GTPase of the subunit and render it “turned on” continuing to stimulate the target. Adenylyl cyclase is one of these targets, and is making cAMP. cAMP has many effects on the interior of the cell, and this mechanism is supposed to be cause to pathology in the organism (Li, 2003).

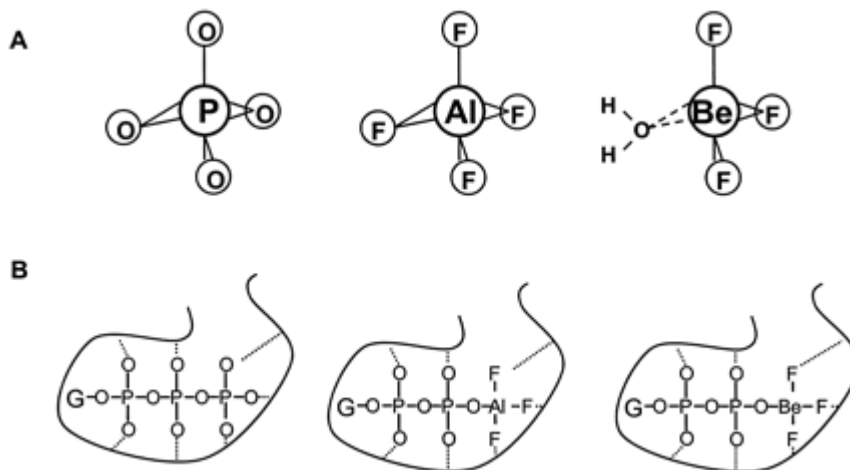


Fig. 12: Similar stereochemistry of AlF_4^- and PO_4^- , and how they fit in the G_{α} subunit.

From <http://cro.sagepub.com/content/14/2/100.figures-only>

Fluoride is also described as affecting the ionic channels on the ER, causing influx of Ca ions to the cytosol. Increase of Ca in the cell also has major effects on the cells enzymes (see Fact box above).

The effects on the signaling pathways may be contradictory and hard to investigate. The endocrine and neurological systems of the body are about communication, humoral and electrical.

Discussion

Fluoride poses a great problem to man in the Eastern African Rift Valley area because of its special volcanic and geologic conditions and nutritional and politicoeconomic conditions. There is a variety of health problems caused by fluoride, but also some potential benefits. Fluoride is a double edged sword.

As pointed out by Gutierrez, most research is done in cell cultures or in animals with very high doses of fluoride, doses that will never be seen by the organism in nature, or even by acute toxicity. To transfer these toxic effects to the effects seen with chronic exposure to elevated levels in e.g. drinking water, is incorrect. Some of the concentrations that cell cultures are incubated in are a thousand fold the concentration seen in the organism, e.g. mg/l levels of fluoride instead of 14 μ g/l as seen in plasma. And test animals are given hundreds of times the concentration seen in natural, high level drinking water, e.g. 300 ppm vs. 3 ppm (300 mg/l vs. 3 mg/l). Such extremes are bound to be toxic, or have extreme effects on the cell or organism.

Some researchers have used physiologic, or near-physiologic concentrations and often seen the opposite effects (Varner et al., 1998, Gutiérrez et al., 1994). Gutiérrez et al.'s experiment

found increased phagocytosis in neutrophils incubated in closer to physiologic levels of fluoride, that is 1-4 μM . That is the opposite of what one would expect, as Houdijk et al. (1991) (according to Gutowska et al. (2012)) found that macrophage's ability to migrate and phagocytize is reduced with high levels of intracellular cAMP. That is if we suppose that fluoride levels and cAMP is closely related as Li (2003) and Gutowska et al. (2012) takes for granted. The mechanism for increased cAMP production with fluoride is widely accepted, but it is unclear if this is valid in vivo or at physiological levels.

In the parts about the pathways the effects of fluoride on function are uncertain, as the pathways' nature is to regulate and compensate each other's actions. AlF_x may stimulate G proteins, but both kinds, the stimulatory and the inhibitory G proteins. What the outcome will be seems uncertain, and is probably different for each cell type. The concentration and even the presence of AlF_x inside the cell are uncertain. One mechanism to raise concentration in vivo could be a concentrating effect in the microclimate at the border of the Ca rich apatite (Li, 2003).

The clinical conditions caused by fluoride on the teeth and skeleton are very well documented and many mechanisms are found as very probable, but some things are still uncertain. There is dispute to the effects fluoride has in low concentrations, and the mechanisms behind the assumed positive effects we see. As an example is the controversy and difficulties of treating osteoporosis with fluoride supplements.

The conclusion of WHO is that fluoride should be added to the drinking-water to a concentration of 1-1,5 $\mu\text{g/l}$ in temperate climates. This should be lower in climates of higher temperature and thereby higher consumption of water. This public health measurement is to decrease the prevalence of caries and its effectivity and cost effectiveness is unprecedented. There is, however, a scientific debate because of the adverse effects and lack of knowledge of the effects in low levels, e.g. the effects on AD. There is also a large movement of more or less conspiracy theories trying to stop this measure. Bahrami (2010) claims the "regular" conspiracies: that the government adds fluoride because of strong corporate powers and to reduce the population's intelligence and cognitive capabilities so they accept the tyranny that is the government. And there are several internet pages lobbying to stop fluoridation: <http://www.fluoridealert.org/> , <http://www.fluoridation.com/> , <http://www.nofluoride.com/> , <http://www.fluoridedebate.com/>. These sites have very unbalanced argumentation. One should discuss whether it is needed, and which approach to take to experience the effects with a minimum of side effects. Norway concluded with not adding fluoride to the drinking-water, and rather focused on information on the use of fluoridated dental products.

There are many suggestions of the effects on the endocrine system. High values of PTH is seen in persons exposed to chronic elevated levels of fluoride, probably due to the influence on the Ca metabolism, what importance this has on the effects seen in skeletal fluorosis is unclear. Fluoride interfering with the iodine metabolism or thyroid function has, after detailed studies in humans, been ruled out, according to Krishnamachari (1987). Influence on the effects on insulin has been indicated and more research on this area would be interesting. Not at least to look for eventual correlation to the problems with metabolic syndrome. As developing countries develop, get more people out of poverty and give them opportunity to buy foodstuff that they earlier could not afford, one sees an increase of overweight and obesity with diabetes type 2 and other associated problems. This would be of importance to these countries in the Eastern Rift Valley and in South-Asia, which also have problems with fluoride.

The effects on the neurologic system are several. In skeletal fluorosis one sees muscle atrophy due to nerve damage, as primary muscle affection has been ruled out. One study has found de- and re-myelinated nerves in biopsies. Neurotoxic effects in the brain have also been seen, although the mechanisms are uncertain. Aluminum fluoride seem to increase the prevalence of AD. But fluoride alone may diminish AD, perhaps by binding to and keep the aluminum from crossing the blood-brain barrier.

Researchers in China (Choi et al., 2012) found that fluoride in drinking water reduced intelligence in children. But they criticized the research going into this systematic review. More research should be done both on the negative effects on and possible protective effects against AD, and on the development in children. Research to find mechanisms for the effects seen could result in treatment methods or find actions against disease promoting.

Fluoride was found to both inhibit and to promote functions of the immune system. Inhibit by inducing oxidative stress and inhibiting immune cell functions such as phagocytosis and migration. The induction of oxidative stress had effect on the histoarchitecture of the spleen and thymus and of decreasing immune cells in circulation. The inhibition of phagocytosis and migration was seen with high intracellular cAMP produced by stimulation from fluoride. Promotion of phagocytosis of bacteria associated with caries, was seen with low doses of fluoride, such found physiologically. This is an example of the biphasic effects of fluoride, and the authors should make it clearer which concentrations are used. Some of the mechanisms are tried to be explained, but it is far from clear. To find out more about the mechanisms at play in the immune cells could again offer possible actions of treating or preventing disease.

Whether inhibition or stimulation, it would be very interesting to find if fluoride could have effects on the immune system that would contribute to the profile of diseases seen in this area, tuberculosis and HIV as examples. Could fluoride exposure be a co-effect to the immune suppression seen with HIV? Or make people more susceptible to the HIV virus or tubercle bacteria? If such a connection is found I would suppose that funding for research on fluorosis would be multiplied.

Li (2003) writes convincingly on the importance of AlF_x as the main active fluoride species in the body. How these complexes affect the phosphoryl transfer reactions and G proteins in cellular pathways. He may be right, but there lacks evidence that AlF_x is even present in the cells interior and whether the concentrations are sufficient to affect the actions of the signal transducers.

If there should be an effect on the G proteins, the outcome would be hard to predict, as each cell type's function differs and the distribution of and effect on inhibitory and stimulatory G proteins may differ.

One question that has arisen is why only the osteoblasts react with proliferation, and not, say osteoclasts or other cells. Research on the mitogenic mechanisms and in which cell types these mechanisms would have effect would be interesting.

Why is there so much problems with fluoride in northern Tanzania and the Eastern Rift Valley area? Several possible reasons can be mentioned.

The conditions for causing high concentrations are discussed above and summarized here:

The arid climate causes limited dilution and refilling of the aquifers, leaving long contact time between the water and minerals. High temperature causes increased solubility, and evaporation and concentration of fluoride. Alkaline volcanic activity carrying rock with minerals containing fluoride. The alkalinity in form of presence of $NaHCO_3$ raises the solubility of fluoride and removes Al which could have formed complexes that precipitated.

Low concentrations of Ca and Mg cause avoidance of precipitation of fluoride complexes. The occurrence of explosive volcanic eruptions with ash and lahar causes presence of easily leachable fluoride containing minerals to the water bodies. Water samples from the area show increased levels of fluoride (table 1).

In addition to high levels of fluoride in the drinking water, the nutrition plays an important role according to several researchers. Ingestion of food containing Ca, Al and phosphate, e.g. from proteins, may inhibit the absorption of fluoride from the intestines (Jha et al., 2011). Malnutrition is a problem and low BMI is a significant predictor of outcome of fluoride exposure. The staple diet for many in the area is “ugali”, a maize-based porridge made with drinking water. Vegetables and meat are, for many, irregular food. Tea has also been found to be a contributor of fluoride excess in this area. Another factor is the magadi salt, made from evaporation of water from a salt water lake. The salt crust that results from the evaporation is used as food flavor and tenderizer and unfortunately contains very high concentrations of fluoride (Jarvis et al., 2013).

The water sources play an important part. Jarvis et al. (2013) speculated in that the older people in their research area in northern Tanzania have less dental fluorosis due to that they got wells 30 years ago. This led to a sudden increase of use of fluoride rich ground water, as they had used low-fluoride containing surface water before that.

Many of the people in this area are of Maasai tribal origin, and traditionally they are cattle herders and live off milk. It would be interesting to see how and whether fluorosis is expressed in those of these people that live in this traditional way, as milk has low fluoride and high calcium content.

In the report “Fluoride in drinking water” by Chilton et al. (2006) there is an excellent part on the different methods used to remove fluoride from the drinking water. The methods are also compared against each other. Bone charcoal, as briefly mentioned is just one method of many. To conclude any actions to be taken for the Eastern Rift Valley or northern Tanzania in general could be unwise. The water samples show great variation in fluoride concentration in different water sources, and without knowing what people drink in each area, a preventive action could be wasted or harmful. “Soft” measures such as nutrition or to speculate with timing of exposure could maybe contribute to less pronounced symptoms, but for me to have opinions about this without knowing more about the local conditions, culture or tradition would be arrogant. More research in collaboration with local authorities and several disciplines such as geology, anthropology, political history, medicine and nutritional science should be undertaken to find good solutions adjusted to the local conditions and people’s needs. In the literature there is not a lack of details, but a lack of connecting those details to a context so that other disciplines can use them as well.

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Lists

List of abbreviations

Adenosine triphosphate – ATP
Aluminum – Al
Aluminumfluoride – AlF
Alzheimer’s disease - AD
Beryllium – Be
Ca²⁺/calmodulin-dependent Proteinkinase – CaM-kinase
Calcium – Ca
Carbon – C
Cyclic adenosine monophosphate – cAMP
Diacylglycerol – DAG
Endoplasmatic reticulum – ER
Figure – Fig.
Fluorine – F
Guanosine diphosphate – GDP
Guanosine triphosphate – GTP
Hydrogen – H
Hydroxide – OH⁻
Immunoglobuline G - IgG
Inositol triphosphate – IP3
Insulin-like growth factor 1 – IGF-1
Lipoxygenase 15 – LOX-15
Magnesium – Mg
Mitogen-activated protein kinase – MAP kinase
Oxygen – O
Parathyroid hormone – PTH
Phospholipase A2 – PLA2
Protienkinase C – PKC
Rough ER – rER
Sodium – Na

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