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EFFECTS OF FLUORINE ON THE HUMAN FETUS

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SUMMARY: In an endemic fluorosis area, 16 fetuses that were delivered during their sixth to eighth month of gestation by means of artificial abortion were collected and studied. The results [compared to 10 control fetuses from a non-endemic area] show that fluorine levels in tissues are obviously high, especially in brain, calvarium, and femur. The activity of alkaline phosphatase in femur and kidney was raised. By observation of the ultrastructure of samples, the number of mitochondria, rough-surfaced endoplasmic reticulum, and free ribosome in neurons of cerebral cortex were reduced, and the rough-surfaced endoplasmic reticulum was obviously dilated. These findings indicate that the neurons of the cerebral cortex in the developing brain may be one of the targets of fluorine.

[Keywords: Acid phosphatase; Adenosine triphosphatase; Alkaline phosphatase; Artificial abortion; Brain; Calvarium; Cerebral cortex; Coal burning; Dental fluorosis; Endoplastic reticulum; Epiphyseal plate; Femur; Fluoride; Kidney; Mitochondria; Microtubules, Neurons; Periosteum; Skeletal fluorosis; Succinic dehydrogenase; Vesicles; Xingwen and Pengshui, Sichuan province, China.]

INTRODUCTION

Fluoride is [widely considered to be] a life-supporting trace element, functioning primarily as protection against dental cavities and also playing a role in bone mineralization. However, excess fluoride can be harmful to organisms. In recent years, researchers have noted that fluoride poisoning appears to begin in the fetal stage.[1,2] Our study collected specimens from induced abortions in both fluoride endemic areas and non-affected areas and, by means of histochemical analysis, enzyme-chemical analysis, light microscopy, and electron microcopy, investigated the effects of fluoride on the fetus, providing evidence for early childhood contraction of fluorosis.

MATERIALS AND METHODS

1. *Source of fetal specimens:* Xingwen and Pengshui counties in Sichuan Province are in high, mountainous areas. Residents use coal with 180–1850 ppm fluoride for warmth and cooking purposes, leading to an epidemic of fluoride poisoning characteristic of coal burning related exposure. The specimens were drawn from these two counties. Each of the mothers of the 16 fetus showed symptoms of dental fluorosis, and 87% had clinical skeletal fluorosis (stage I to III). Their staple food was corn contaminated with 18.5–88.5 ppm fluoride, and there were no signs of rickets or other diseases of skeletal metabolism. The control specimens came from Chengdu, which has low water and food fluoride, and each mother was healthy. In both groups, the fetuses were aborted at 6–8 months.

2. *Determination of tissue fluoride levels:* Fresh tissue was collected and incinerated in a box furnace. A UJ-25 DC potentiometer and a FHS-2 PH meter

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were then used together to determine fluoride content as per the standard additive method.

3. *Histochemical analysis:* Tissue specimens were taken from the bone, liver, and kidneys and subjected to standard tissue enzyme-chemical techniques, testing for the levels of AKP [alkaline phosphatase], ACP [acid phosphatase], SDH [succinic dehydrogenase], and ATPase [adenosine triphosphatase] activity using semi-quantitative methods.

4. *Light microscopy:* Heart, liver, lungs, kidneys, brain, and bone tissues were examined using standard methods, with HE [hematoxylin-eosin] and VG [van Gieson] staining.

5. *Electron microscopy:* Brain and femur samples were taken, placed in a 2.5% glutaric dialdehyde solution, and prepared for standard electron microscopy (the bone samples were first decalcified using EDTA). The samples were then inspected and pictures taken using H-600 and H-300 transmission electron microscopes.

RESULTS

I. FLUORIDE CONTENT OF FETAL TISSUES: (see Table 1)

Table 1. Fluoride levels (ppm) in various tissue samples from endemic and control fetuses

Group	n	Thymus	Heart	Liver	Lungs	Kidney	Brain	Muscle	Placenta	Cartilage	Femur
Endemic	16	50.5	50.1	45.5	40.3	39.9	31.7*	53.3	36.8	50.9	129.8*
Control	10	48.1	47.4	43.4	40.1	40.0	23.2	49.1	38.2	41.9	60.5

* P<0.05

From Table 1 it is clear that no matter which group is considered, the average tissue fluoride is highest in the bone tissue and lowest in brain tissue. Both femur and brain fluoride were markedly higher in the endemic group as compared with the control, and the difference is significant (P<0.05). The differences in all other tissues groups are not significant at P>0.05.

II. ENZYME AND TISSUE CHEMISTRY:

As compared to the control group, the activities of AKP and ACP on and around the femur trabeculae of the fluorosis endemic area fetuses were more pronounced, while the SDH and ATPase activity in the kidneys were slightly low, and the AKP activation slightly high. The activity of AKP, ACP, ATPase, and SDH in the liver showed no significant differences.

III. LIGHT MICROSCOPY:

1. *Cerebral cortex:* Under the light microscope, there were no visible differences in the tissue structure or the size or morphology of the nerve cells in the endemic area fetuses as compared to the control, nor were there any signs of pathological changes.

2. *Femur:* Comparison of the femurs of the endemic and control area fetuses, the epiphyseal plate of the former showed disorderly arrangement and clustering of the cartilage cells, the trabeculae near to the epiphysis varied greatly in thickness,

the calcification of the metaphyses and diaphyses were incomplete, and there were few ossified cells on the surface; only a few of the cartilage stroma of the stained trabeculae had retained calcified cartilage cells. The periosteum and the elastic fibers had thickened, and the fibers were bunched. The arrangement of the collagen fibers was disorderly. Under the periosteum of the metaphyses and diaphyses, new bone formations and denaturing of the cartilage stroma were visible (Figure 1). The controls exhibited none of the above changes, and their morphology showed no irregularities.

IV. ELECTRON MICROSCOPY:

1. *Cerebral cortex:* Nerve cells within the cerebral cortex of the endemic fetuses contained fewer mitochondria, granular endoplasmic reticula, and ribosomes than the controls; the mitochondria showed marked swelling, the granular endoplasmic reticula had expanded, and the nuclear membranes were damaged, with the contents of the nucleus spilling out of the nuclear envelope. Within the nucleus, there was an increase in heterochromatin, with some grouping around the edges. Synapses were relatively rare, and those noted were enlarged, with the synaptic membrane broken and fewer mitochondria, microtubules, and vesicles than usual (Figure 3). In cerebral tissue of the control group, the organelles within the nerve cells were plentiful, with normal mitochondria and granular endoplasmic reticula; the cell structure was undamaged, and there was an abundance of euchromatin in the nucleus (Figure 2).

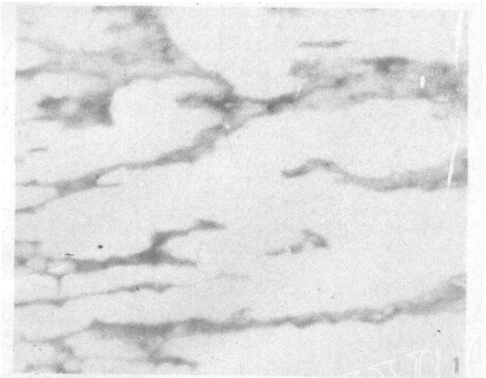

Figure 1. Endemic area: The epiphysis and cartilage cells are on the right in this figure. Trabeculae are stretched to the left with incomplete calcification and disorderly arrangement (Van Gieson, × 1,300).

Figure 2. Control area: Within synapses, there are normal mitochondria, granular endoplasma, and synaptic vesicles. The microtubules and synaptic membranes are clear (× 50,000).

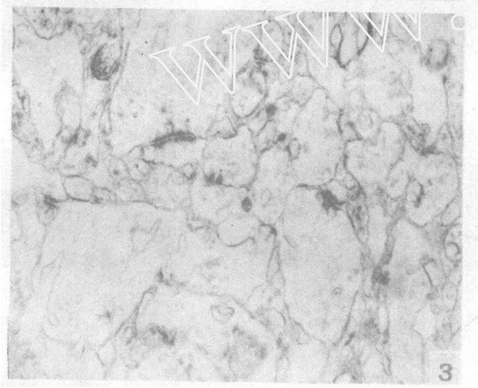

Figure 3. Endemic area: Synapses contain fewer mitochondria, synaptic vesicles, and microtubules (× 40,000).

2. *Femur:* The femurs from the endemic group had relatively few osteoblasts. The osteoblasts that were visible contained more granular endoplasmic reticula

than usual and were clearly expanded and reticulated. There were also more mitochondria, showing signs of enlargement. Moreover, there were a large number of secretory granules both inside and outside the cell membrane. By contrast, the control group had more bone cells with larger nuclei, less cytoplasm, and abundant Golgi bodies. (Figures 5 and 6). The granular endoplasmic reticula, mitochondria, and the collagen fibers around the cells and in the bone stroma of the control cells were well defined and arranged regularly (Figure 4).

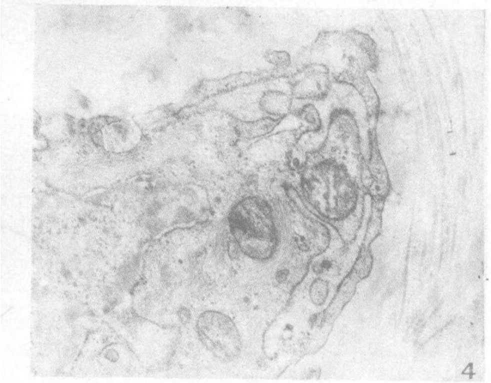

Figure 4. Control area: Osteoblasts on the right and collagen fibers show regular arrangement (× 10,000).

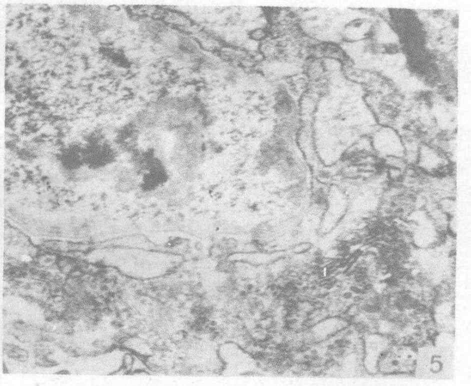

Figure 5. Endemic area: An electron micrograph of osteoblasts. The nucleus is on the lower right. Mitochondria show marked swelling, with less or even loss. Granular endoplasmic reticula are expanded with secretory granules both inside and outside the cell (× 20,000).

DISCUSSION

When the various hard and soft tissues taken from fetuses as part of this study were tested for fluoride, the results showed that the brain and bone tissue of the fluoride endemic area fetuses had higher fluoride content than the controls ($P < 0.05$). The reason for this disparity is the previous excess fluoride intake of the mother, clearly reflected in the medical condition of the mother; 100% of the 16 endemic area mothers suffered from dental fluorosis, and 87% had clinical skeletal fluorosis, indicating a high body load of fluoride that was exacerbated by continued regular ingestion of fluoride contaminated (18.5–88.5 ppm) corn during pregnancy. After the fluoride entered the body of the mother, it passed through the placenta and into the various organs and tissues of the fetus. With 96% of fluoride uptake deposited in bone tissue, the fluoride ions replace the hydroxyl group in the hydroxyapatite found in bones, forming the strongly bonded fluorapatite; fluoride has a special affinity for bone, and so this combination is not easily split apart.

Figure 6. Endemic area: Within the bone stroma, collagen fiber arranged irregularly (× 40,000).

With regard to fluoride in soft tissues, except for the brain, there was no significant difference between the endemic and control groups, i.e., no sign that fluoride was collecting in these various tissue types. This is likely because the half-life of fluoride in soft tissues is relatively short; in general fluoride levels in soft tissues do not increase as age or time of exposure increases.[3]

Fluoride can pass through the blood-brain barrier and accumulate in brain tissue. Thus in our study the brain tissue of the fetuses from the fluoride endemic area showed higher fluoride levels than the control. The mechanisms involved are not yet clear. Besides increased amounts of fluoride, the brain tissue of the endemic subjects also showed nerve cells with swollen mitochondria, expanded granular endoplasmic reticula, grouping of the chromatin, damage to the nuclear envelope, a lower number of synapses, fewer mitochondria, microtubules, and vesicles within the synapses, and damage to the synaptic membrane. These changes indicate that fluoride can retard the growth and division of cells in the cerebral cortex. Fewer mitochondria, microtubules, and vesicles within the synapses could lead to fewer connections between neurons and abnormal synaptic function, influencing the intellectual development after birth. These questions await further research.

There have also been reports in the literature of fluoride inhibiting the synthesis of DNA and external proteins that promote cell growth. Thus fluoride poisoned rats and their fetal offspring had decreased amounts of RNA in their brain tissue. By competing with citric dehydrogenase, inhibiting succinic dehydrogenase, cytochrome oxidase, and the oxidative phosphorylation process, fluoride creates a barrier to proper energy metabolism.[4-6] Given the results of this study, it can be theorized that after excess fluoride enters the brain, it restricts the synthesis of RNA and forms a barrier to energy metabolism. RNA is directly related to the processes of gene information transcription, translation, and amino acid transport, which in turn make possible the synthesis of proteins. Therefore, a decrease in RNA synthesis and a disruption of energy metabolism would hinder the synthesis of protein and relevant enzymes, leading ultimately to slowed growth, poor cell division, and changes in the ultrastructure of the neural cells.

On the surface of the bone, generally regarded as inactive, there is a layer of pyrophosphate, inhibiting the natural calcification of bone. Alkaline phosphatase (AKP), however, is a pyrophosphate activator, so it can neutralize the calcification inhibiting effect of pyrophosphate. In the fetuses from the endemic area, the present study found that although the activity of AKP near the trabeculae was increased, there was no corresponding increase in ossification; rather, calcification was obviously incomplete. This indicates that the effect of fluoride on calcification is not simply a matter of AKP activity; there must be other factors present.

Susheela et al. have noted a decrease in the synthesis of collagen in the spongy bone matter of fluoride poisoned rabbits, and Messer, Golule, et al. report that fluoride can inhibit decomposition of the collagen fibers in bone. Our own study revealed that the collagen fibers of the femur bone stroma in the fluoride-poisoned fetus tended to pile together, showing deranged, crisscross patterns, with very few osteoblasts. The vast majority of osteoblasts that were present appeared to be lacking in organelles, indicating diminished or inhibited synthesis of collagen. We believe that besides inhibiting the synthesis of collagen proteins, fluoride also hinders the decomposition of collagen proteins, and in fact the latter inhibition is the stronger one, causing an accumulation of collagen fibers, and further influencing bone calcification.

Another factor in the calcification of normal bone matter is the combined effect of collagen and chondroitin sulfate or some other proteoglycan forming a special kind of stereochemical structure that allows an accumulation of bone salts. In the femurs of endemic area fetuses, the collagen in the bone stoma was bunched together and disordered, and thus it could not form this special stereochemical structure, thereby negatively influencing the calcification of the bone.

Under normal circumstances, the growth of bone involves the ossification of cartilage. In the fluoride-affected fetuses, the excess fluoride had a stimulating effect on the periosteum, causing it to thicken and bunch, with disordered elastic and collagen fibers. These changes are the basis for pathological bone formation, including denaturing of the cartilage stroma and formation of new bone under the periosteum.

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