

# Influence of low and high doses of fluoride on tooth germ development in rats

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*The influence of fluoride on tooth germ development, especially mineralised tissue, is well documented in numerous dental publications, but there are few reports concerning the influence of fluoride on enamel organ and dental papilla cells. The aim of the study was to assess histologically the development of tooth germs of 20-day-old rat foetuses whose mothers drank water without fluoride or with low (10 mg) and high (110 mg) contents of sodium fluoride, starting from the 12<sup>th</sup> day of the pregnancy. The fluoride contained in drinking water in low as well as high concentration accelerated the development of enamel organ and dental papilla structures in rat foetuses. The acceleration was proportional to the content of fluoride in drinking water. No disturbances caused by high concentration of sodium fluoride were observed.*

**key words:** tooth germ, development, fluoride, rats, foetus

## INTRODUCTION

Although the role of the fluoride ion in the mineralisation and maturation of dental tissues has been explored for many years, the mechanism of this influence is not completely clear, especially with reference to its cariostatic activity and dental fluorosis.

The fluoride ion can incorporate with dental enamel in stable and mobile ways [3]. Fluoride in the stable form is incorporated directly into the crystalline lattice of enamel prism. In the mobile way fluoride can be present in the organic matrix of enamel, the hydration shell surrounding the prism and in the extracellular fluids. F ions can substitute hydroxyl ions and constitute fluoroapatites with formula  $\text{Ca}_5(\text{PO}_4)_3\text{F}$  [4]. The diameter of F ion is smaller than hydroxyl, so the change of  $\text{OH}^-$  into  $\text{F}^-$  contributes to a better 3-Ca rank and constitutes a better plane of the crystal. F ion is more electronegative than OH ion so the electrostatic attraction between F and  $\text{Ca}^{2+}$  is stronger. Fluoride can also attract protons of adjacent apatite calcium ions. Moreover, the central position of fluoride in the  $\text{Ca}^{2+}$  triangle elim-

inates the former dipole between  $\text{Ca}^{2+}$  and  $\text{OH}^-$ . As a consequence of the facts mentioned above, fluoridated apatite is better crystallised, which means that the crystals are more regular and stable, less soluble in acids and less sensitive to the oral environment. Thus, the general opinion is that the stable part of fluoride directly incorporated into the crystalline lattice reduces caries development and progression [4].

On the other hand, fluoride ions present in high concentration in the organic matrix, extracellular fluids or body fluids can adversely affect the enzymatic protein degradation, which leads to the disturbances of the dental enamel mineralisation and maturation [3]. Neither the level of fluoride concentration nor the mechanism of dental fluorosis induction is discerned. The problem is still subject to investigation. In rats which received acute doses (20 mg/kg b.w.) of fluoride in injections, disturbances in the enamel quality but not appositional growth were observed [15]. In contrast, in roe deer reduction of enamel matrix production was observed as the result of fluorosis [9]. Observations indicate that for

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enamel fluorosis induction the most important is the age of animals, duration of exposition and the total intake of fluoride. Taking into account the fact that the absorption of fluoride in the young organism is higher than in the older one, the aim of the study was to assess histologically the influence of different fluoride concentration in drinking water on the first lower molar tooth germ development in rat foetuses.

## MATERIAL AND METHODS

Three groups of 20-day-old male Wistar foetuses (5 animals per group) were used. All groups received standard diet [12] and drank respectively: the first group distilled water without fluoride, the second group distilled water with 10 mg NaF/l, the third group distilled water with 110 mg NaF/l. The water intake was not measured but regular monitoring showed that water was drunk by all groups and required replenishment daily. On the 20<sup>th</sup> day of pregnancy pregnant females and their male foetuses were killed under ethereal anaesthesia by decapitation. The foetuses heads were fixed in 10% formalin for 24–48 hours. After fixation the tissues were dehydrated and embedded in paraffin. Serial 6  $\mu$ m thick sections were cut in the frontal plane and stained with haematoxylin and eosin. Sections were inspected under the microscope Jenamed 2 Carl Zeiss Jena.

## RESULTS

### Group 1 — control (distilled water without fluoride; Fig. 1A)

The tooth germ development of the first lower molar assumed the bell stage. The junction to dental lamina is reduced to several layers of cells. In the enamel organ the partition into the parts of histologically different cells can be noticed. The most external layer consists of cubical cells which form outer enamel epithelium. The most internal layer of the enamel organ, called inner enamel epithelium is composed of high columnar ameloblasts. Between these two epithelia is found the middle width enamel pulp. Enamel pulp is built from not numerous star-shaped cells connected with each other with desmosomal junctions. The space between cells is filled with mucoid fluid. Between enamel pulp and ameloblasts, stratum intermedium is not so distinctly expressed. In the dental papilla the most external layer of cells, directly connecting with ameloblasts, is formed from cells histo-differentiated into odontoblasts, but the layer of odontoblasts is not well pronounced.

### Group 2 — (distilled water with 10 mg NaF per litre; Fig. 1B)

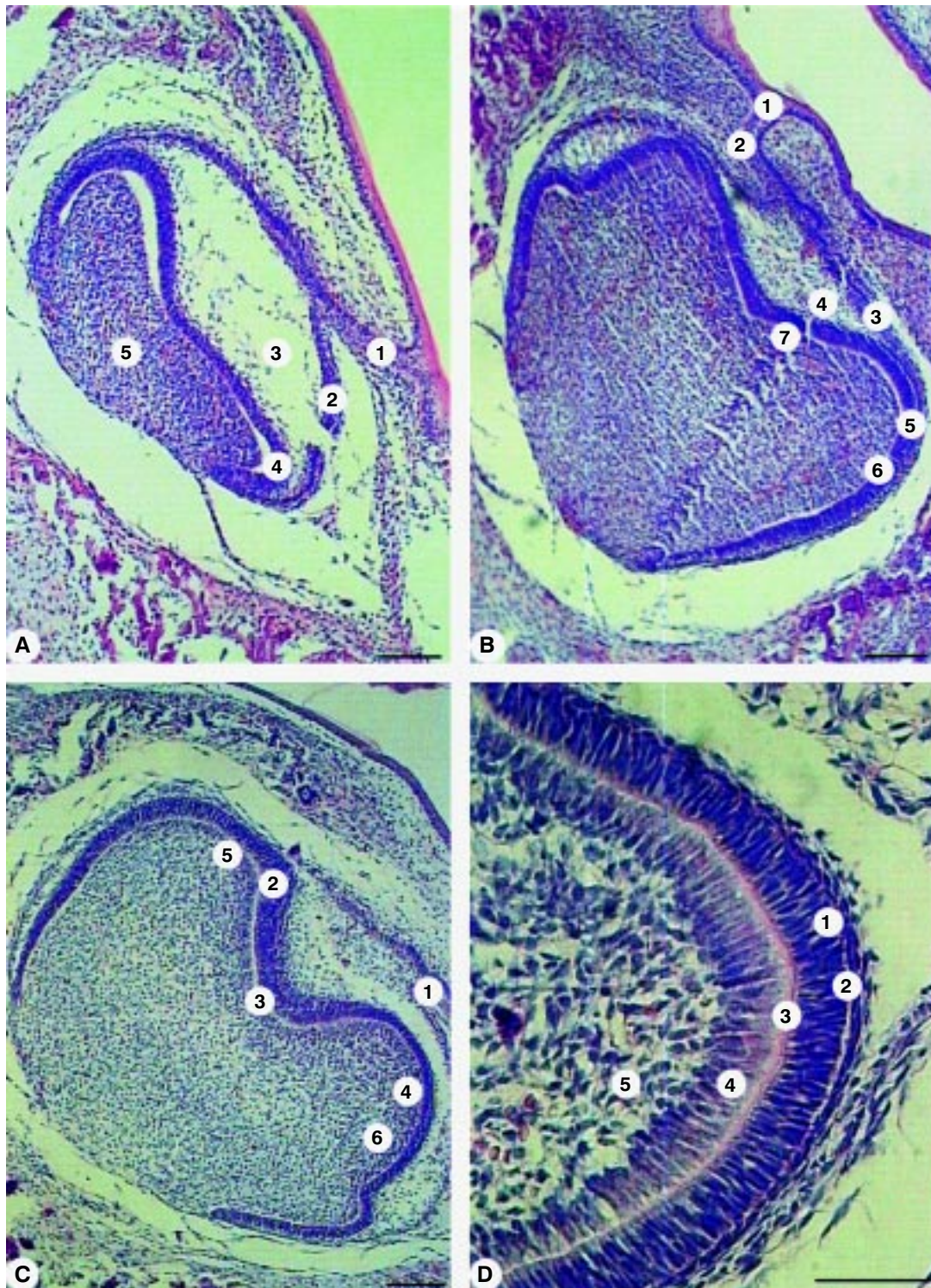
The tooth germ development of the first molar in mandible assumed the late bell stage. The junction with dental lamina is almost cut off. In the enamel organ three different layers of completely histo-differentiated cells are discerned. In the outer enamel epithelium not numerous budding blood vessels can be noticed. The enamel pulp is strongly reduced. Ameloblasts form a distinctly expressed layer of cells. Their nuclei are moved to the basic parts of cells. The several layers of squamous cells adjacent to ameloblasts form stratum intermedium. In the dental papilla a well-pronounced layer of columnar odontoblasts is seen. The dentino-enamel junction is distinctly expressed.

### Group 3 — (distilled water with 110 mg NaF per litre; Fig. 1C,D)

The development of first molar in mandible assumed the crown stage and completed histo-differentiation. The junction with the dental papilla is definitely cut off. The enamel organ consists of the outer epithelium formed from the single layer of cubical cells with numerous budding blood vessels. The enamel pulp is completely reduced. Ameloblasts form the single layer of cells directly connecting with the dentino-enamel junction. Their nuclei are situated in the basic parts of cells. On the opposite side of ameloblasts there is the stratum intermedium composed of several layers of squamous cells. Odontoblasts completed their histo-differentiation and the deposition of the first layer of predentin can be noticed. In the dental pulp there are numerous budding blood vessels seen between fibroblasts.

## DISCUSSION

For years fluoride has been known as the most common caries prevention factor. The role of fluoride as a caries reduction factor is still being discussed [6,15]. In the maturation stage of enamel development, non-toxic fluoride concentration accelerated amelogenins degradation with the increase of the Ca<sup>2+</sup> transport [3]. High concentration of fluoride directly, adversely affects ameloblasts secretion activity and inhibits protease activity, thus retards amelogenins degradation [1,2,10,14]. Fluoride in low doses is effective in caries prevention only when low carbohydrate diet is applied simultaneously [15]. Fluoride affects the late secretion and early maturation stages of odontogenesis because the maximum fluoride enamel deposition takes place during early



**Figure 1.** The morphological picture of the lower molar tooth germ in mandible of 20-day-old rat foetus belonging to A) group 1; B) group 2; C) group 3. The sections stained with HE. (Scale bar = 0.1 mm).

Arabic numerals in figure **A**) indicate: 1 — junction with dental lamina, 2 — outer enamel epithelium, 3 — enamel pulp, 4 — ameloblasts, 5 — dental pulp. **B**) 1 — oral epithelium, 2 — junction with dental lamina, 3 — outer enamel epithelium, 4 — enamel pulp, 5 — ameloblasts, 6 — dentin – enamel junction, 7 — odontoblasts. **C**) 1 — broken junction with dental lamina, 2 — ameloblasts, 3 — dentin – enamel junction, 4 — apposite layer of predentin, 5 — odontoblasts, 6 — dental pulp. **D**) The morphological picture of the cusp of lower molar tooth germ in mandible of 20-day-old rat foetus belonging to group 3. Arabic numerals in figure D indicate: 1 — ameloblasts, 2 — stratum intermedium cells, 3 — predentin, 4 — odontoblasts, 5 — dental pulp. The section stained with HE. (Scale bar = 0.05 mm).

phases of odontogenesis [8,10,14,16]. Lyarman et al. [11] observed that fluoride affected only the secretory stage but never the organic matrix apposition. Aoba [4], in contrast, observed also the suppression of the enamel matrix production. The increased number of lysosome-like structures in ameloblasts cytoplasm suggests the hypothesis that the synthesis of abnormal products in ameloblasts cells are induced by the high concentration of fluoride [13]. In our study we observed the more advanced maturation of inner enamel epithelium, ameloblasts and odontoblasts in rat foetuses whose mothers drank water with low and high concentration of natrium fluoride. In our experiments, fluoride accelerated the first lower molar tooth germ development in rats. In groups given water with low and high dosages of natrium fluoride, the histo-differentiation of each component of enamel organ and dental papilla was more advanced. This was particularly observed in the enamel organ maturation in which we could observe the increased number of budding blood vessels in the layer of outer epithelium. The decreased quantity of the enamel pulp observed in rats which drank water with high concentration of natrium fluoride indicates that nutrition supply for ameloblasts was being reduced. The movement of ameloblasts nuclei into the basic parts of cells, observed in groups that drank water with low and high fluoride contents, confirmed that ameloblasts were ready for enamel deposition. The first layer of predentin was observed only in the group which drank water with high concentration of natrium fluoride. The broken junction with dental lamina indicates that the lower first molar tooth germ finished the histo-differentiation and undertook the functions of dentin and enamel deposition. Our observations agree with those of Glenn et al. [7], who observed that the development of enamel organ and dental papilla in human foetuses whose pregnant mothers took fluoride tablets was more advanced than in controls. Our results concerning the dentin are in opposition to Appleton's [5]. He noticed the high irregularity in predentin and unmineralised spaces in the dentin of weaning rats on 0.05% and 0.1% fluoridated diet. In our study we did not observe any disturbances in predentin caused by high concentration of natrium fluoride in drinking water

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