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Effects of diet and fluoride on early phases of odontogenesis in rats

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The influence of diet and fluoride on odontogenesis in rats was investigated. 20 foetuses, 20 days old, were divided into four groups. The control group was fed with the standard diet and drank water with 0.16 mg F/l. The second, third and fourth groups were fed with the deficit, experimental diet and drank distilled water with 10 and 110 mg of natrium fluoride per litre or without fluoride. In each group, the observed tooth-bud development assumed different stages. The less advanced cap stage assumed the tooth-bud in the group fed with the deficit diet and given distilled water without fluoride. In the remaining groups, the development of observed first molars in mandible assumed the different level of its advancement in the same stage of odontogenesis — bell stage.

key words: odontogenesis, tooth-bud, teeth, histodifferentiation, dentin, enamel, enamel organ, dental pulp

INTRODUCTION

The initiation of the development of rat's first molar in mandible begins on the 13th day of prenatal life. Exogenous and endogenous factors are able to modify numerous stages of odontogenesis. Particular phases of the tooth-bud growth and maturation, like initiation, proliferation, histodifferentiation, as well as the apposition of dentin and enamel, are stimulated with different strength by exogenous factors like diet and fluoride.

The proper proportion between proteins, lipids and carbohydrates in a pregnant rat's diet is a very important factor in peculiar tooth-bud development of rats' foetuses. The dietary requirements for laboratory animals increase during pregnancy and intensive maturation of young animals. In this case the required level of albumin increases up to 20–24% and lipids up to 2–8%. Farris and Griffith [13] have synonymously confirmed the stoppage of rats' growth after the reduction of albumin in diet up to 10%. Malnutrition can become the reason for disturbances in odontogenesis. The low fatty acids diet provides a pleomorphic picture in odontoblasts, seen as picnotic nuclei and vacuolisation in cytoplasm. Large free spaces are formed between predentin and dentin as well as between odontoblasts and predentin [23]. The diet not only exerts a direct influence on the body's development and maturation, but also modifies the interaction between other compounds of diet, for example trace elements and poisons, so a synergetic or antagonistic correlation may be observed.

The reservoir of fluoride in the body is bones and teeth. The quantity of fluoride assimilation depends on the kind of applied diet [17,18,20]. The starvation, high lipid diet, and tropical climate increase the fluoride absorption, while the high protein, low lipid diet, rich in magnesium, calcium and

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C-vitamin distinctly decrease fluoride absorption and toxicity [17,24]. Fluoride exerts the greatest influence on calcium – phosphoric equilibrium. The greatest intensity of fluoride setting in hard tissues of teeth, takes place during their highest mineralisation [8–10].

In the present study we tried to estimate how natrium fluoride dissolved in drinking water in two doses (high 110 mg NaF/l and low 10 mg NaF/l) and the low protein, low lipid diet applied at this same time influenced the development of rats' foetuses' first molar in mandible.

MATERIALS AND METHODS

Four groups of 20–dayold male Wistar foetuses (5 animals per group) were used. In our experiment the first (control) group received a standard diet (Table 1) calculated on the basis of Szczygiel et al.'s and Wysocki's tables [28,31], and drank water with 0.16 mg F/l. The second, third and fourth groups were fed with Keyes Experimental Diet 2000 (Table 2) [16] which was a deficit diet and watered respectively with: the second group distilled water, the third group distilled water with 10 mg NaF/l, the fourth group distilled water with 110 mg NaF/l. In every group the animals drank about 250–400 ml water per day. On the 20th day of pregnancy under ethereal anaesthesia pregnant females and their male foetuses were killed 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 μm thick sections were cut in the frontal plane and stained with haematoxylin and eosin as well as with azan after Heidenhain. The sections were inspected under a microscope Jenamed 2 Carl Zeiss Jena.

RESULTS

Group 1 — control (standard diet + water with F as a trace element equal to 0.16 mg per litre; Fig.1A). The tooth-bud development of first molar in the mandible assumed the medium bell stage. The connection with dental lamina is reduced to a few layers of cells. In the enamel organ the outer and inner

Table 1. The composition of the standard diet [28,31]

Products of the diet	Capacity in 100 g of the diet [g]	Capacity in 100 g of the diet [g]		
		proteins [g]	lipids [g]	carbohydrates [g]
Ground oat	22.0	25.0	6.7	50.0
Ground wheat	20.0			
Ground rye	20.0			
Meat-bone flour	15.0			
Desiccated ground yeast	6.0			
Powdered milk	5.0			
Desiccated lucerne	5.0			
Desiccated ground casein	4.5			
Cod liver oil	1.0			
Calcium carbonate	1.0			
Salt	0.5			
TOTAL:	100.0			

Table 2. The composition of the deficit diet — Keyes Experimental Diet no. 2000 [16]

Products of the diet	Capacity in 100 g of the diet [g]	Capacity in 100 g of the diet [g]		
		proteins [g]	lipids [g]	carbohydrates [g]
Powdered sugar	56.0	15.7	2.4	75.1
Powdered defatted milk	28.0			
Ground wheat	6.0			
Powdered lucerne	3.0			
Desiccated ground liver	1.0			
Salt	2.0			
Desiccated brewer's yeast	4.0			
TOTAL:	100.0			

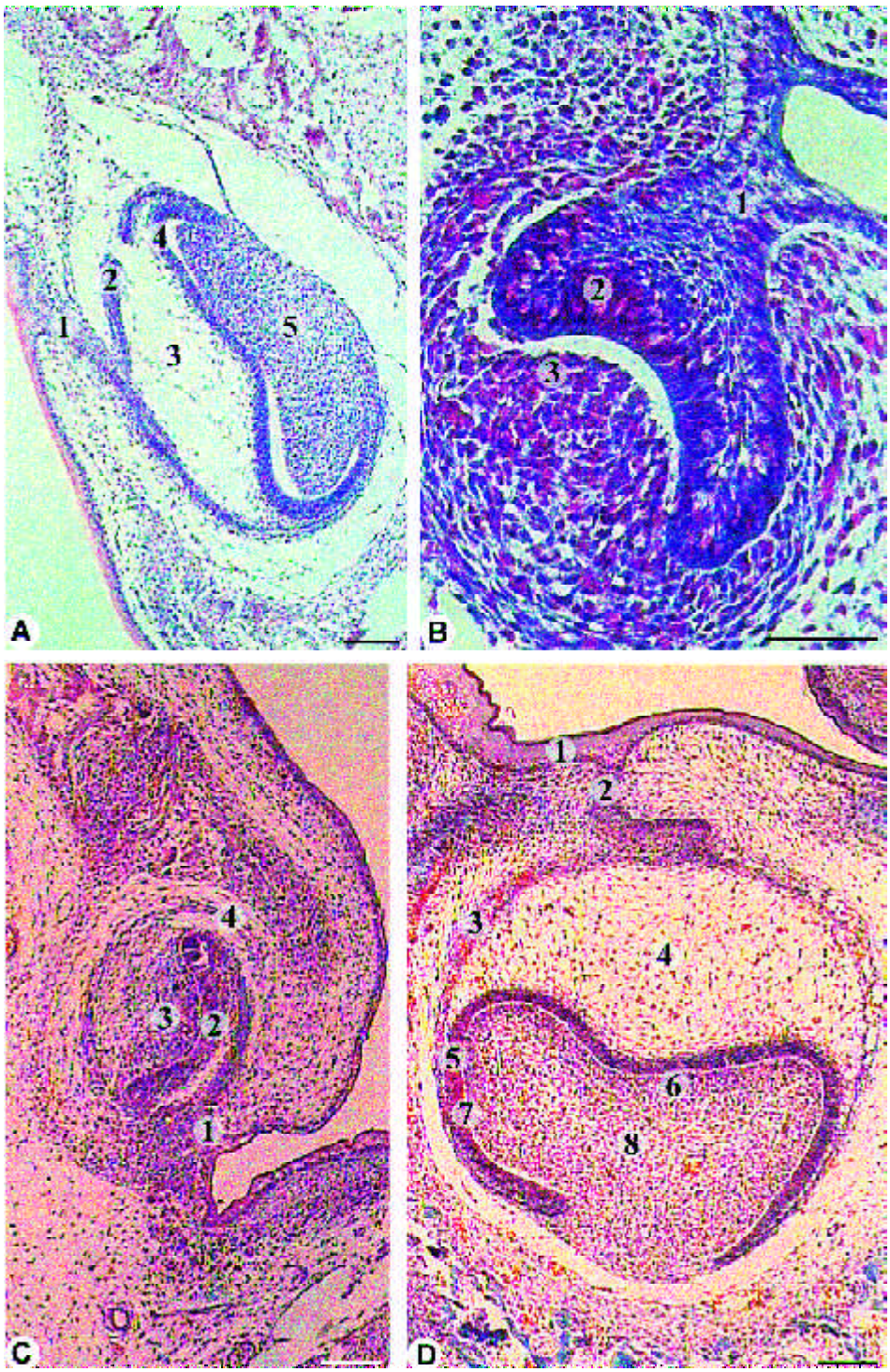

Figure 1. The morphological picture of the first lower molar tooth-bud of the 20-day-old rat's foetus belonging to the:

A) control group — 1. The junction with the dental lamina (1), the outer epithelium of the enamel organ (2), the enamel pulp (3), ameloblasts (4), the dental pulp (5). The section stained with HE. Scale bar — 0.1 mm.

B) group — 2. The junction with the dental lamina (1), the enamel organ (2), the dental papilla (3). The section stained with HE. Scale bar — 0.05 mm.

C) group — 3. The junction with the dental lamina (1), the enamel organ (2), the dental pulp (3), the dental sac (4). The section stained with HE. Scale bar — 0.1 mm.

D) group — 4. The dental lamina (1), the almost broken junction between the enamel organ and the dental lamina (2), the outer enamel epithelium with gemmating blood vessels (3), the enamel pulp (4), ameloblasts with nuclei placed in the basic parts of cells (5), odontoblasts (6), the enamel — dentin border (7), the dental pulp (8). The section stained with azan after Heidenhain. Scale bar — 0.1 mm

epithelia as well as stellate reticulum are seen. The inner epithelium at the concavity of the enamel organ consists of a single layer of tall cells — ameloblasts. The nuclei of ameloblasts start to move to the basic parts of cells. Several layers of squamous cells appear between the inner epithelium and the enamel pulp, and form the stratum intermedium. The enamel pulp is of middle width and consists of cells which assume reticular form, giving a characteristic network with spaces filled with mucoid fluid. The outer epithelium consists of a single layer of cubical cells. The mesenchyma of the dental papilla largely enclose into the invaginated part of the enamel organ. The layer of cells directly connecting with the inner enamel epithelium has become histodifferentiated into high, columnar form – odontoblasts, but the layer of odontoblasts is not distinctly expressed. The tooth–bud is surrounded by condensed mesenchyma, called the dental sac.

Group 2 — (experimental diet + distilled water without fluoride; Fig. 1B). The tooth-bud development of first molar in the mandible assumed the cap stage. The connection with the dental lamina is wide. In the enamel organ unequal, expand growth in marginal parts lead to invagination of central part of above. The division into three parts in the enamel organ can be seen. These are the inner epithelia, outer epithelia and reticular enamel pulp. Directly under the organising enamel organ, the mesenchyma compress and invaginate into the enamel organ to form dental papilla.

Group 3 — (experimental diet + distilled water with 10 mg NaF per litre; Fig. 1C). The tooth-bud development of first molar in the mandible assumed the early bell stage. The connection with the dental lamina is reduced to several layers of cells. The cells coming from the enamel organ are divided into three parts. These are: the inner epithelia, outer epithelia and the reticular enamel pulp. The inner epithelium consists of a single layer of tall, columnar ameloblasts. The enamel pulp is formed from a little volume of mucoid fluid and numerous cells. There is no stratum intermedium. The outer epithelium is created from a layer of cubical cells. The mesenchyma of the dental papilla is enclosed into the invaginated part of the enamel organ. The most exterior layer of mesenchymal cells begins histodifferentiation into tall odontoblasts. The tooth-bud is surrounded by condensed mesenchyma, called the dental sac.

Group 4 — (experimental diet + distilled water with 110 mg NaF per litre; Fig. 1D). The tooth-bud development of first molar in mandible assumed the advanced bell stage and finished histodifferentiation. It seems it could almost be classified as the early crown stage. The connection to the dental lamina is almost cut off. The enamel organ is divided into three parts of histologically different cells. These are: a single layer of cubical cells forming the outer epithelium in which the gemmation of non-numerous blood vessels can be seen. The enamel pulp is very wide because of the increase of mucoid fluid. Cells of the enamel pulp are star-shaped with long processes, which anastomose each other. The inner enamel epithelium consists of a single layer of tall, columnar ameloblasts. The nuclei of ameloblasts are situated in the basic parts of cells. The most exterior layer of the dental pulp is formed from cells, which have assumed a columnar form – odontoblasts. Between the ameloblasts and the odontoblasts the dentino–enamel junction is distinctly expressed, which is important for the morphodifferentiation of the dental crown. The tooth-bud is surrounded by compressed mesenchyma, called the dental sac.

DISCUSSION

Malnutrition in prenatal life, as well as in the growing up and maturation period, is the reason for abnormalities in the structure and functions of organisms [12,14]. The more important the dietary element which is restricted, the bigger the abnormalities which may be observed in all the body's development, including in the structures of the teeth [15,27,30]. The present study showed the strong link between deficient diet and slower odontogenesis. In group 2, where animals were fed with the deficient, experimental diet and drank distilled water without fluoride, the proliferation of cells in the enamel organ was distinctly slower. Consequently the development of first molar in mandible assumed the cup stage in the 20th day of prenatal life. The enamel organ multiplies specially in marginal parts. The predisposition to division into three parts can be noticed but without specific features of cells in a particular area. In mesenchyma, connected with the enamel organ, the strong condensation of cells is observed but also without histodifferentiation. In control group 1, in which animals were fed with the standard diet, the development of examined tooth-bud assumed the medium bell stage. The histodifferentiation in all enamel structures and in dental papilla is very advanced. The results of the present study are conformable to those obtained by Pawlak et al. [21,22] and Adamowicz-Klepalska et al. [1–3]. These authors have described the development of

rats' periodontium in adequate experimental conditions.

Malnutrition modifies the reaction of the organism to other dietary elements, for example toxic compounds. It is still crucial to establish the influence of fluoride and its derivatives for human beings and animals. In the present study the low doses (10 mg/l) of natrium fluoride accelerated the development of the examined tooth-bud in comparison to group 2, in which animals drank water without natrium fluoride. The tooth-bud development in group 3 assumed the early bell stage but the cause of the deficient diet was not as advanced as in control group. In group 3 advanced histodifferentiation in the enamel organ was observed but in the mesenchymal dental papilla it was distinctly less marked. Because of the inductive affect of the inner epithelium of the enamel, in the most peripheral parts of the dental papilla minimal disposition to histodifferentiation into odontoblasts can be seen. There is still no stratum intermedium.

Many investigators tried to establish the part of odontogenesis, which is most sensitive to the toxicity of fluoride. It was shown that the late secretory phase and early enamel maturation were most sensitive [4–7,11,19,25,26,29]. Fluoride in high doses blocks the enzymatic degradation of amelogenins and it breaks the growth of enamel prism crystals. This is the reason for immature enamel formation. At the same time stimulation of calcium precipitation abates the level of free calcium mineralisation zone. It is possible that the alteration in solubility of enamel fluoroapatites disturbs the diffusible equilibrium of calcium and other ions present in the liquid zone surrounding the prism. Monsour et al. [19] observed the inhibition in the growth of enamel prism crystals in rats' teeth after intraperitionel injection of 20 mg NaF, but he has not noticed any disturbances in apposition of the enamel. It suggests that the intra-prismatic substance must have been deposited in the enamel of explored teeth. Appleton [7] has described changes in rats chronically intoxicated with 0,1% NaF in diet: a large interglobular area in the dentin, the wide layer of the predentin, irregular border between predentin and dentin, sometimes there was no border at all. The apposition of the dentin was slower in comparison to the control group. In the present study high doses (110 mg/l) of natrium fluoride in drinking water accelerated the observed tooth-bud development, although animals were fed with deficient diet. In group 4 the completion of the histodifferentiation was observed in the enamel organ and dental papilla as well. All cells were completely prepared for their function. The tooth-bud development transgressed calendar age. In our experiment low as well as high doses of natrium fluoride in drinking water accelerated the teeth-bud development in rats' foetuses, equalising the retardation caused by malnutrition and no disturbances caused by high dosages of natrium fluoride were noticed.

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