한국전자현미경학회지 제 36 권 제 1 호 2006
Korean J. Electron Microscopy 36(1), 17~23(2006)

Dose-dependent Ultrastructural Changes of the Odontoblasts in the Neonatal Rat after Ingestion of the Fluoride to the Pregnant Rat

Moon-Jin Jeong¶, Soon-Jeong Jeong¶ and Do-Seon Lim[1,*]
Department of Oral Histology, College of Dentistry, Chosun University, Gwangju
[1]Department of Dental Hygiene, Seoul Health College, Sungnam, 461-713, Korea
(Received February 13, 2006; Accepted March 23, 2006)

음용수 불소농도에 따른 흰쥐태아 상아모세포의 미세구조적 변화

정 문 진¶, 정 순 정¶, 임 도 선[1,*]
조선대학교 치과대학 구강조직학교실, [1]서울보건대학 치위생과

ABSTRACT

The pregnant rats were given a drinking water administration of the sodium fluoride and normal saline for control animals. The sodium fluoride produced cellular changes of odontoblast with consistent response. Compare to control group, the odontoblasts that were administrated by sodium fluoride, showed significantly ultrastructural differences including large number of free ribosomes and swelled mitochondria in dose-dependent manner (300 ppm).

From fine structural and morphological investigations of the changes in odontoblast, there were three distinctive structural changes: (1) destruction of the endoplasmic reticulum, (2) swelling of the mitochondria, and (3) severe cellular derangement of the endoplasmic reticulum and mitochondria. From this consecutive structural change, we observed that sodium fluoride temporarily affects the cell organelles in odontoblasts (100, 200 ppm), suggesting it is important that optimal concentration of the sodium fluoride in developing fetus of the rat.

Key words : Fluoride, Neonatal rat, Odontoblasts, Pregnant rat, Ultrastructural changes

INTRODUCTION

Dentin, the dense calcified tissue that forms the bulk of the tooth, is broadly similar to bone in composition but is more highly mineralized and thus much harder than bone (Linde & Goldberg, 1993). The cells responsible for dentin formation, the odontoblasts, differentiate as a

¶ These two authors equally contributed to this work.
* Correspondance should be addressed to Dr. Do-Seon Lim, Department of Dental Hygiene, Seoul Health College, Sungnam, 461-713, Korea. Ph.: 031-740-7229, FAX: 031-740-352, E-mail: idsun@sh.ac.kr

single layer of tall columnar cells on the surface of the dental papilla apposed to the ameloblast layer of the enamel organ.

The fully differentiated odontoblast is a highly polarized, secretory cell responsible for the deposition of proteinous dentin matrix (Sasaki & Garant, 1996). Earlier work on odontoblasts *in vitro* has been concentrated with detailed studies on development (Glasstone, 1967), epithelial-mesenchymal interaction associated with morphogenesis and differentiation of tooth germs (Slavkin & Bringas, 1976). If odontoblast is affected by several physiological factors (internal and external) during dentinogensis, it comes with no developing to normal dentin.

It is well established that in many species the exposure of developing dentin and enamel to sodium fluoride, both in vivo, as a dietary additive or by subcutaneous or intra-peritoneal injection (Elsenmann & Yaeger, 1969; Fejerskov et al., 1979). A previously published report on the effect of the sodium fluoride on the odontoblast and dentin indicated that 8.4 mg of this effect on several morphological changes in the incisor of the adult rat (Araki, 1989), however, these studies only have limited to adult rat with observation of the dentin.

Using developing neonatal rat, the response of the odontoblasts to dietary additive has not been demonstrated until now. According to these reasons, we tried to identify the morphological changes affected by dose dependent manner of the sodium fluoride. In addition the present study was to investigate determine whether fluoride has an effect on odontoblast in developing neonatal rat. Moreover we have tried to find out optimal concentration of the sodium fluoride because this study is the first report on the developing fetus of the rat.

MATERIALS & METHODS

Sprague-Dawley rats dieted with water containing 0ppm (group I), 100 ppm (group II), 200 ppm (group III), 300 ppm (group IV) of the sodium fluoride at the beginning of the pregnancy. After delivery 12-day old postnatal rats (20～25 gm) were anaesthetized with Ether, then per-fused with 4% paraformaldehyde and/ or 2% glutaraldehyde in sodium cacodylate buffer, pH 7.4. The jaws were removed and placed for pre-fixation in the same medium of the perfusion fixation. They were washed in sodium cacodylate buffer. To remove organic remains from the enamel surface, the teeth were carefully washed in distilled water, treated with a 3% solution of the hypochloride for 5 min, rinsed in distilled water and finally treated with ultrasonic waves for 5 min. They dehydrated through a graded series of ethanol.

For transmission electron microscopy, the specimen was embedded in Epon Mixture via propylene oxide. Polymerization was carried out at 60°C for 48 hours. For routine histology, semi-thin sections were cut out on glass knives, mounted on glass slide, and stained with 1% toluideine blue in 1% borax. Ultra-thin sections were obtained from a LEICA ultramicrotome using diamond knives. The sections were mounted on 200 mesh nickel grids, and double stained with a uranyl acetate and lead citrate. The sections were examined with a HITACHI H-7600 electron microscope at 80 kV.

RESULTS

Fluoride was administrated to rats in varying doses (100, 200, 300 ppm) in order to study the likelihood of ultrastructural alterations to odontoblasts and dentin as well. Fetuses of the rat from day 11 old, incisors were examined by the light and transmission electron microscope with particular attention to intra-tissue organization and extracellualr matrix.

In light microscopic observation, odontoblasts layer of the control animal was observed as a single row of cells at the periphery of the pulp on the inner aspect of dentin (Fig. 2-1, 2). Fine structural analysis showed that odontoblasts were tall, columnar-like cells with basal nuclei, much granular endoplasmic reticulum, and a large sup-

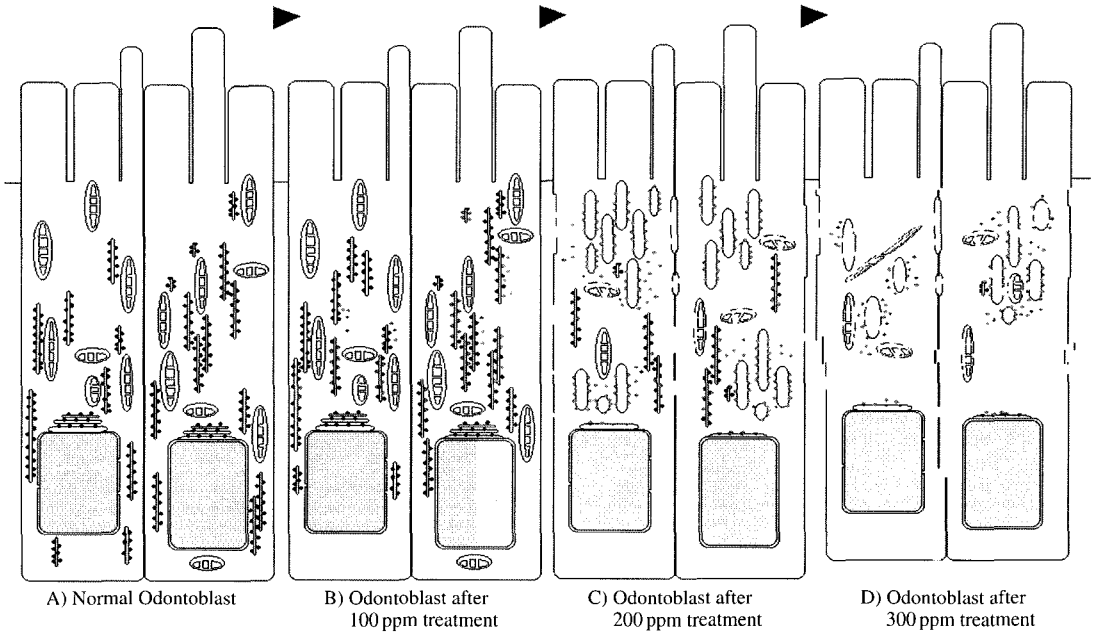

Fig. 1. Diagram of the progression of the odontoblasts cellular changes.

ranuclear Golgi apparatus (Fig. 2-3, 4).

The intercellular spaces observed in 100-ppm dose of the sodium fluoride were less conspicuous and the odontoblasts looked like those in the control group (Fig. 3-1, 2). In the cells there was a degranulation of the rough-surfaced endoplasmic reticulume (Fig. 3-3, 4).

At 200-ppm, enlarged intercellular spaces similar to those in the 100-ppm group were seen between the odontoblasts (Fig. 4-1). The endoplasmic reticulums were significantly decreased in number as compared with those after treated 100-ppm sodium fluoride. Characteristic changes in the odontoblasts layer appeared in areas, which corresponded to the cytoplasmic vacuolizations (Fig. 4-2, 3, 4).

In 300-ppm administration, the odontoblasts were apparently reduced in size and in the amount of their cell organelles as compared with the previous stage (Fig. 5-1, 2). At this stage, the detached ribosomes were remarkably increased in number. We also observed that destroyed mitochondria were wrapped with endoplasmic reticulum in this dose of sodium fluoride (Fig. 5-3, 4). All about the progression of cellular changes were well represented figure 1.

DISCUSSION

Fluoride has long been recognized to provide one of the best public health measures in the prevention of dental caries (Ferjerskov et al., 1996), in addition to having a potential role in the prevention of osteoprorosis (Hillier et al., 1996). The concentration of fluoride that cells are exposed to appears to be a critical factor in determining any changes in behavior that may occur. It has been known that below 1 ppm is normal concentration to digest directly on the adult rat (Kortelainen & Larmas, 1993). However, from previous report it is difficult to determine whether fluoride has an indirect effect on the odontoblasts and ameloblasts in the developing fetus of the rat. Through the preliminary experi-

ments to the developing fetus (using 30, 50, 70, 90 ppm dietary), we have identified that a range of concentration for cellular changes: there were no cellular changes in all concentrations (data not shown).

According to the studies of the fluoride effects on the amelobalsts, there were several changes of organelles including swelling and destruction of mitochondria, expanding of the rough endoplasmic reticulum after injection of the fluoride (Kruger, 1970; Appleton, 1994). In this study, severe fine structural alterations and collapse of the odontoblasts shape were observed in 300 ppm treated groups. In 100 and 200 ppm groups, though the organelles of the odontoblasts were slightly affected by fluoride, there was no destruction of the cells. On the based previous reports, therefore, it is common structural changes after injection of fluoride (Walton & Eisenmann, 1975; Appleton, 1988). Moreover, we confirmed and suggested that these changes of the organelles are the criteria to the extent of injury in both odontoblasts and amelobalsts on the fluoride ingestion.

The present study has allowed investigation of the indirect-effects on the fetus of the sodium fluoride exposure at defined concentrations. Thus, this study provides additional information that complement previous studies on the effect of fluoride on the adult rat (Takano & Ozawa, 1980; Salama et al., 1991). Furthermore, our results demonstrated that 100 and 200 ppm of sodium fluoride shows reversible injury and 300 ppm occurs irreversible damage to the odontoblasts, suggesting that under the 200 ppm of fluoride is optimal concentration to the developing fetus on the rat.

ACKWLEDGEMENT

This study was supported by research funds from Chosun University, 2006.

REFERENCES

Appleton J: The ultrastructure of dentine from rat incisors following exposure to sodium fluoride and potassium pyroantimonate staining. Scan Micros 2(4) : 2045-2054, 1988.

Appleton J: Formation and structure of dentine in the rat incisor after chronic fluoride. Scan Microsc 8 : 711-719, 1994.

Araki S: Ultrastructural changes in rat-incisor odontoblasts and dentin caused by administration of sodium fluoride. Shikwa Gakuho 89(1) : 49-91, 1989.

Elsenmann DR, Yaeger JA: Alterations in the formation of rat dentin and enamel induced by various ions. Archs Oral Biol 14 : 1045-1064, 1969.

Ferjerskov O, Yaeger JA, Thylstrup A: Microradiography of calcified tissue. Int Rev Cytol 56 : 183-253, 1979.

Ferjerkskov O, Ekstrand J, Burt BA: Fluoride in dentistry. 2nd Ed Copenhagen, 1996.

Glasstone S: Development of teeth in tissue culture. J. Dent. Res., 46 : 858-861, 1967.

Heywood BR, Appleton J: The ultrastructure of the rat incisor odontoblast in organ culture, Archs. Oral Biol 29(4) : 327-329, 1984.

Hillier S, Inskip H, Coggon D, Cooper C: Water fluoridation and osteoporotic fracture. Commun Dent Health 13 : 63-68, 1996.

Kortelainen S, Larmas M: Effect of fluoride on caries progression and dentin apposition in rats fed on a cariogenic or non-cariogenic diet. Scand J Dent Res 101 : 16-20, 1993.

Kruger BJ: The effect of different levels of fluoride on the ultrastructure of amelobalsts on the rat. Arch Oral Biol 15 : 109-114, 1970.

Linde A, Goldberg M: Dentinogenesis. Crit Rev Oral Biol Med 4(5) : 679-728. 1993.

Sasaki T, Garant PR: Structure and organization of odontoblasts. The Anatomical Record 245 : 235-249, 1996.

Salama AH, Zaki AE, Eisenmann D: Fine structural changes and lysosomal phosphatase cytochemistry of ameloblasts associated with the transitional stage of enamel formation in the rat incisor. Am J Ant 190 : 279-290, 1991.

Slavkin HC, Bringas P: Epithelial-mesenchyme interactions during odontogenesis. IV. Morphological evidence for direct heterotypic cell-cell contacts. Devl Biol 50 : 428-442, 1976.

Takano Y, Ozawa H: Ultrastructural and cytochemical observations on the alternation morphologic changes of the ameloblasts at the stage of enamel maturation. Arch Histol Jpn 43 : 385-399, 1980.

Walton RE, Eisenmann DR: Ultrastructural examination of dentine formation in rat incisors following multiple fluoride injections. Arch Oral Biol 20 : 485-488, 1975.

<국문초록>

음용불소가 흰쥐태아의 상아모세포에 간접적으로 미치는 영향과 농도를 확인하기 위해 임신한 흰쥐에 100, 200, 300 ppm의 불소가 함유된 음용수를 임신기간 동안 투여하였다. 대조군과 비교하였을 때, 농도에 의존적으로 상아모세포의 미세구조적 변화를 관찰 할 수 있었다: 조면소포체로 부터 리보솜의 탈락과 붕괴, 미토콘드리아의 팽창, 그리고 조면소포체와 미토콘드리아의 변성. 음용 불소의 농도에 따른 미세구조적인 변화로부터 100, 200 ppm에서는 가역적인 그리고 300 ppm에서는 비가역적인 손상이 유도되는 것을 확인하였다. 또한, 이러한 결과로 미루어 200 ppm 미만의 농도가 발생중인 흰쥐태아에 영향을 주지 않는 것을 알 수 있다.

FIGURE LEGENDS

Fig. 2. Control incisor of the neonatal rat.
1, 2. Odontoblasts (OB) are attached to the predentin (PD) and dentin (D). The odontoblasts are the predominant cell type. 3. The distal portion of odontoblast has numerous endoplasmic reticulum and mitochondria. Also odontoblast contains numerous endoplasmic reticulum and secretory vesicles of the low electron density. Scale bar=2.3 μm. 4. The proximal portion of the odontoblast with a basally displaced nucleus (N) and associated endoplasmic reticulum (arrows), Golgi apparatus (G) and mitochondria (M). The Golgi region occupies the center of the cells and is surrounded by profiles of the rough endoplasmic reticulum. Adjacent odontoblasts are tightly attached together by numerous adherence junctions. Scale bar=0.3 μm.

Fig. 3. Incisor after ingestion of drinking water containing 100-ppm sodium fluoride.
1, 2. The intercellular spaces are less conspicuous and the odontoblasts look like those in the control group. The odontoblasts are tall columnar but of somewhat uneven length. 3, 4. The vacuoles are most prominent in three parts of the cells, namely proximally to the nuclei, close distally to the nuclei and in the most distal part of the cells. At the ultrastructural level, these vacuoles appeared to be swollen and disrupted mitochondria (M). In the same cells there are a disintegration and degranulation of the rough-surfaced endoplasmic reticulum (arrows) and of the poly-ribosomes. Respectively, Scale bar=2.3 μm, Scale bar=0.8 μm.

Fig. 4. Incisor after ingestion of drinking water containing 200-ppm sodium fluoride.
1. Enlarged intercellular spaces, similar to those in the 100-ppm group, are seen between the odontoblasts. These seem to increase in size and number with increasing doses of sodium fluoride. There were a number of destroyed cell fragments both in the predentin and pulp under the injured dentin. 2. Swelled rough-surfaced endoplasmic reticulum and mitochondria. Scale bar=2.3 μm. 3, 4. Endoplasmic reticulum (arrows) are decreased in number as compared with those after treated 100-ppm sodium fluoride. Characteristic changes in the odontoblast layer appeared in areas, which corresponded to the cytoplasmic vacuolization. Respectively, Scale bar=0.3 μm, Scale bar=0.4 μm.

Fig. 5. Incisor after ingestion of drinking water containing 300-ppm sodium fluoride.
1, 2. Slender cells possessing high N/C ratios and prominent nucleoli and irregular-shaped cells are observed in the degenerating odontoblast layer. 3, 4. Odontoblasts are apparently reduced in size and in the amount of their cell organelles as compared with the previous stage. The detached ribosomes were remarkably increased in number. Cytoplasm of the odontoblast is still morphologically different from that of a normal functional odontoblast in that the profiles of the intact rough-surfaced endoplasmic reticulum are few in numbers and there are numerous apparently free ribosomes in the cytoplasm. Destroyed mitochondria are wrapped with endoplasmic reticulum (arrow). Scale bar=0.3 μm.

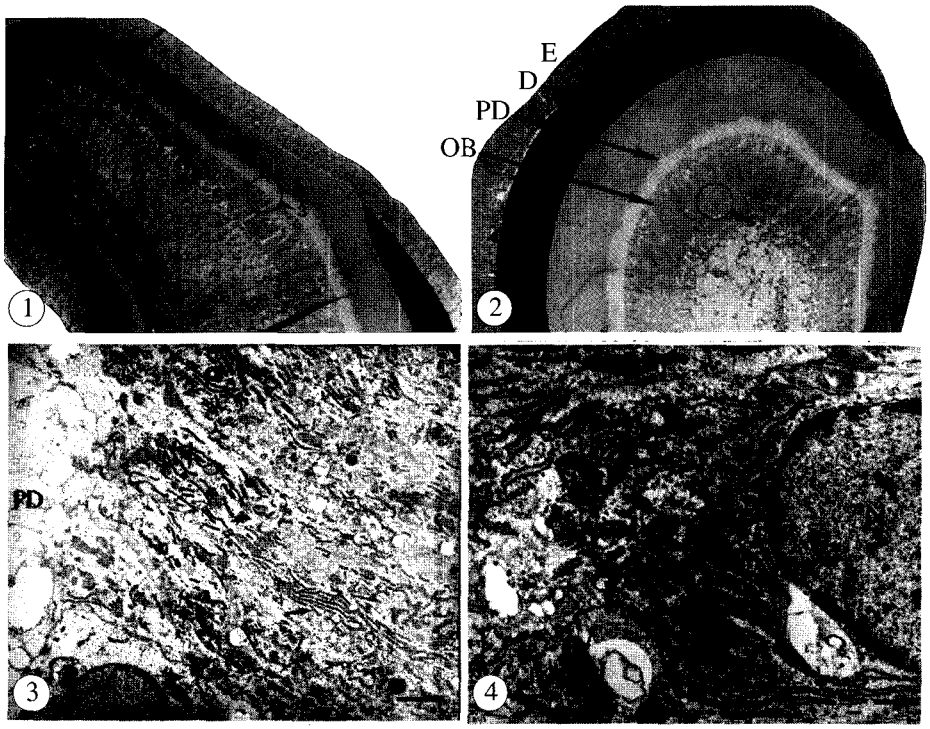

Fig. 2

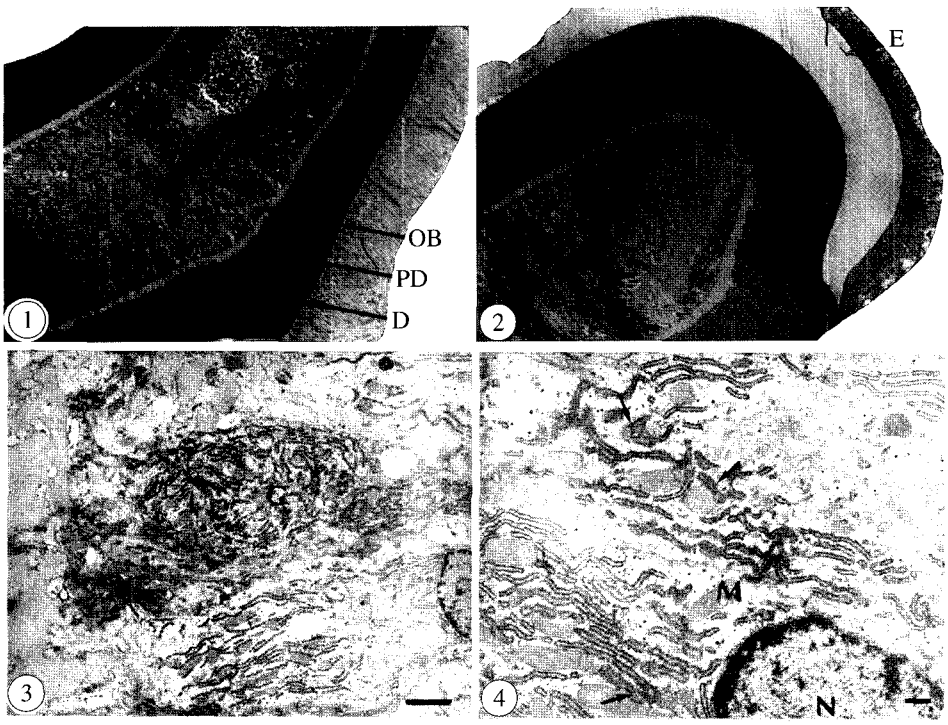

Fig. 3

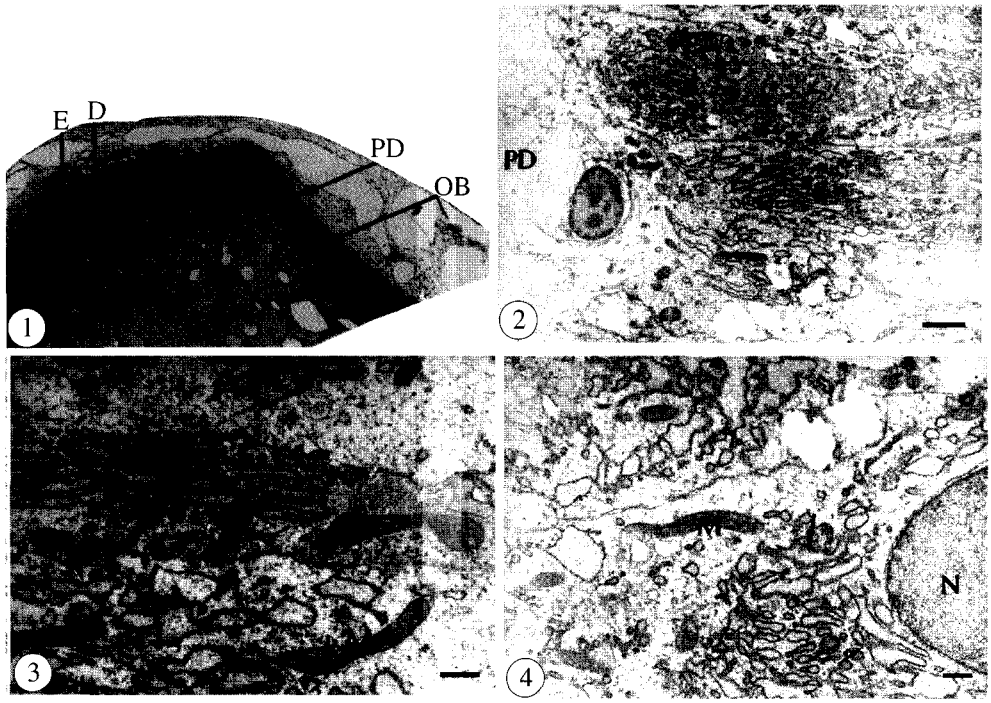

Fig. 4

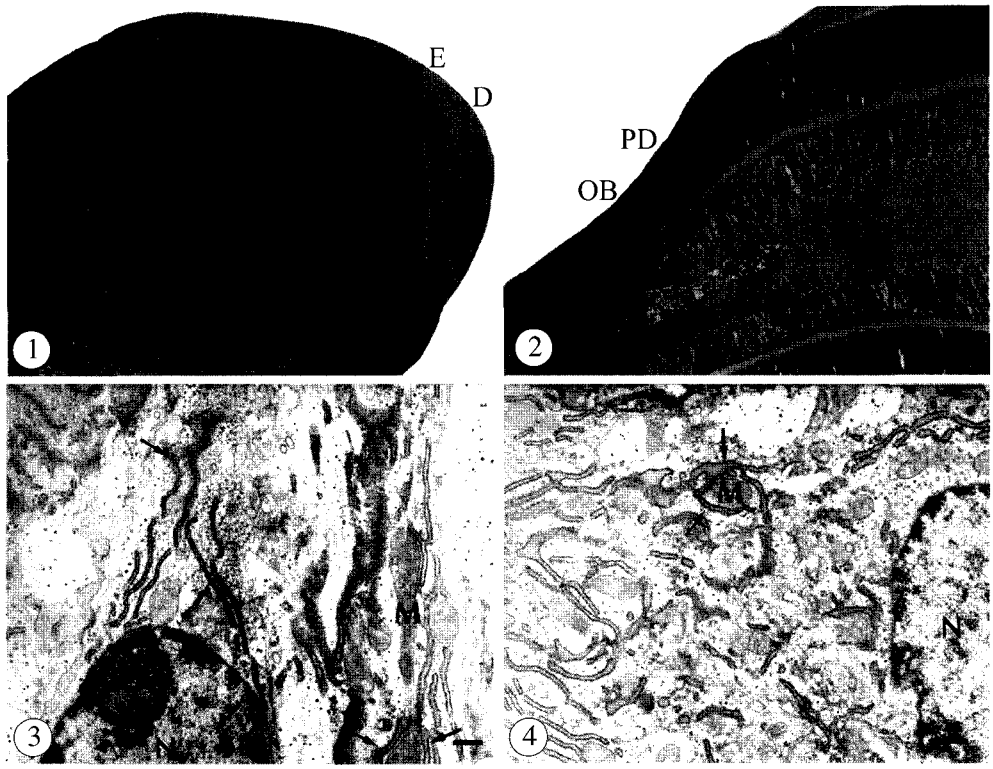

Fig. 5