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Ultrastructural study of osteodentin formation induced by irradiation

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SUMMARY : **A single dose of 16,000 rads was delivered to a 1 x 1.5 cm area of the cheek of 2 day old Wistar rats. The lower first molar region was removed 1, 3, 5 and 8 days post-irradiation and processed for light and electron microscopy observation.**
The most distinct structural feature at optical level was osteodentin production in the dental papilla, which began at the level of the initiation of Hertwig's epithelial sheeth and continued until the pulpal area was completely filled by osteodentin.
At the ultrastructural level, ameloblasts exhibited large lipidic vacuoles and irregular intracytoplasmic secretory granules. Odontoblasts underwent multiple regressive alterations with vacuolization and numerous myelin figures. Pulpal cells exhibited a capacity for odontoblastic differentiation with formation of one or more odontoblastic processes and dentin matrix secretion. Thus, irradiation resulted in stimulation of a great potential for dentin production in these cells.

KEY WORDS : *Osteodentin - Irradiation - Tooth germ.*

RESUME : **Une dose unique de 16 000 rads a été appliquée sur une surface de 1 x 1,5 cm de la joue des rats Wistar, âgés de deux jours. La région de la première molaire inférieure, prélevée 1, 3, 5 et 8 jours après l'irradiation, a été étudiée en microscopie optique et électronique. La formation d'ostéodentine dans la papille dentaire a été la caractéristique structurale la plus évidente en microscopie optique. Cette formation débute au niveau de l'initiation de la gaine épithéliale de Hertwig et l'apposition se poursuit jusqu'à ce que la totalité de la région pulpaire soit remplie par l'ostéodentine. En microscopie électronique, les améloblastes présentent de larges vacuoles lipidiques et des granules de sécrétion intracytoplasmiques irrégulières. Les odontoblastes subissent diverses altérations régressives avec des vacuolisations et de nombreuses figures myéliniques. Les cel'ules de la pulpe présentent une capacité de différenciation odontoblastique avec formation d'un ou plusieurs prolongements odontoblastiques et sécrétion matricielle de dentine. Ainsi l'irradiation semble stimuler les grandes potentialités de production de dentine de ces cellules.**

MOTS CLES : *Ostéodentine - Irradiation - Germe dentaire.*

ZUSAMMENFASSUNG : **Mit 16 000 rad als Einzeldosis wurde ein 1 x 1,5 cm grosser Bezirk der Wange von 2 Tage alten Wistar-Ratten bestrahlt. Die Region im Bereich des unteren ersten Molaren wurde 1, 3, 5 und 8 Tage nach Bestrahlung reseziert und zur licht- und elektronenmikroskopischen Untersuchung vorbereitet. Die auffallendste strukturelle Veränderung war bei optischer Betrachtung die Osteodentinproduktion in der Zahnpapille, welche im Keimzentrum der Hertwig' Epithelscheide begann und sich fortsetzte, bis der gesamte Pulpabezirk durch Osteodentin ersetzt war. Ultrastrukturell entfalteten Ameloblasten ausgedehnte Lipid-Vakuolen und unregelmässige intrazytoplasmatische, sekretorische Granula. Odontoblasten wurden zahlreichen regressiven Veränderungen mit Vakuolisierung und Myelinablagerung unterworfen. Pulpazellen entfalteten die Fähigkeit, Odontoblasten mit Entwicklung von ein oder mehreren Odontoblastenfortsätzen und mit der Abscheidung von Dentinmatrix zu differenzieren. Die Bestrahlung stimulierte bei diesen Zellen deshalb die Dentinentwicklung.,**

INTRODUCTION

Experimental irradiation of tooth germs produces regressive changes in amelogenesis and dentinogenesis. Hypoplastic lesions in the enamel, niche-shaped recesses in dentin formation, reduction in the thickness of predentin and dentin and degeneration of odontoblasts have been the most frequently described features after irradiation with doses ranging from 200 to 5,000 rads (Leist, 1925 ; Smith, 1938 ; English and Tullis, 1951 ; Adkins, 1968 ; Koppang, 1969 ; Lindvall et al., 1972). In addition to these regressive changes, formation of pulpal osteodentin has also been consistently reported.

The abnormal dentin was at first called « depolarization dentin » because it was thought to be produced by depolarized odontoblasts (Pliess and Franke, 1960 ; Koppang, 1967). In later studies, at light microscopy level, Adkins (1967) and Koppang (1973) suggested that the pulpal mesenchymal cells could differentiate into osteoblast like cells producing osteodentin. The capacity for osteodentin formation has been observed even after irradiation with doses as high as 20,000 rads, which cause ulceration of the oral mucosa and skin and severe injury to the ameloblasts and odontoblasts (Cabrini et al., 1967 ; Manfredi et al., 1971).

The purpose of the present work was to extend the aforementioned observations to the ultrastructural level and to observe the cytological aspects of the osteodentin producing cells after stimulation by high doses of irradiation.

MATERIAL AND METHODS

A localized beam of X rays was used to irradiate the molar region of 25 newborn Wistar rats. A single dose of 16,000 rad was delivered through rectangular 1 x 1.5 Pb diaphragms using a Philips radiotherapy unit operated at 250 kv and 13 mA, employing a 0.25 mm Cu and 1 mm Al filter at a dose rate of 500 rad/min. Non-irradiated littermates were kept as controls.

Groups of 12 animals were killed 1, 3, 5 and 8 days post-Irradiation.
The left mandible of 3 irradiated and 3 control animals from each group were formalin-fixed, decalcified in 5 % EDTA at pH 7, for 15 days and processed for routine paraffin embbeding, sectioning and Hematoxylin-eosin stain.

The remaining 3 experimental and 3 control animals from the same group were used for electron microscopy observations. The lower left first developing molar was extracted and fixed in cold Karnovky fixative (1965) for 2 hr, washed in phosphate buffer (pH 7.2) for 2 hr, and decalcified in EDTA-TRIS solution (pH 6.9) at 4°C for one week (Fullmer and Link, 1964). After decalcification, the tooth germs were washed again in phosphate buffer and sectioned into 3 mm thick slices in a central bucco-lingual plane. The slices were post-fixed in 1 % OsO_4 and flat-embbeded in Epon 812 to mantain adequate orientation during sectioning.

Ultrathin sections were mounted on copper grids, and stained with uranyl acetate and lead citrate, and examined in a Philips 200 electron microscope.

RESULTS

Optical microscopy

No changes were detected at the optical level 24 hr after irradiation. From 3 days post-irradiation onwards, alterations in the odontoblastic layer were evidenced by an irregular arrangement of more deeply stained cells.

Apposition of osteodentin also became visible 3 days after irradiation. In all specimens studied, osteodentin formation begun in the proliferation zone of the pulp near the initiation of Hertwig's epithelial sheeth. By the 5th day, osteodentin formation extended beneath the whole odontoblatic layer. Eight days after irradiation, the entire pulp area showed a diminished cellularity and deposition of an eosinophilic homogeneous material **(Fig. 1).**

No ameloblastic alterations were observed in the light microscopy preparations at any of the periods post-irradiation studied.

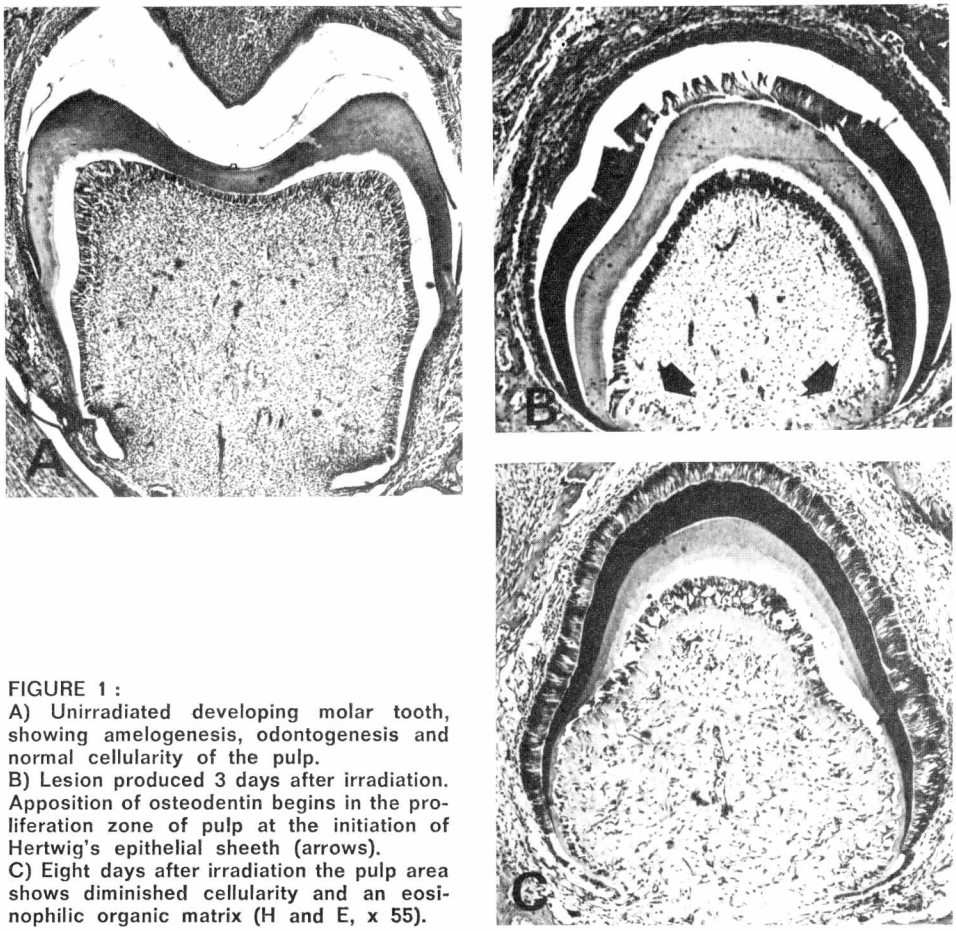

FIGURE 1 :
A) Unirradiated developing molar tooth, showing amelogenesis, odontogenesis and normal cellularity of the pulp.
B) Lesion produced 3 days after irradiation. Apposition of osteodentin begins in the proliferation zone of pulp at the initiation of Hertwig's epithelial sheeth (arrows).
C) Eight days after irradiation the pulp area shows diminished cellularity and an eosinophilic organic matrix (H and E, x 55).

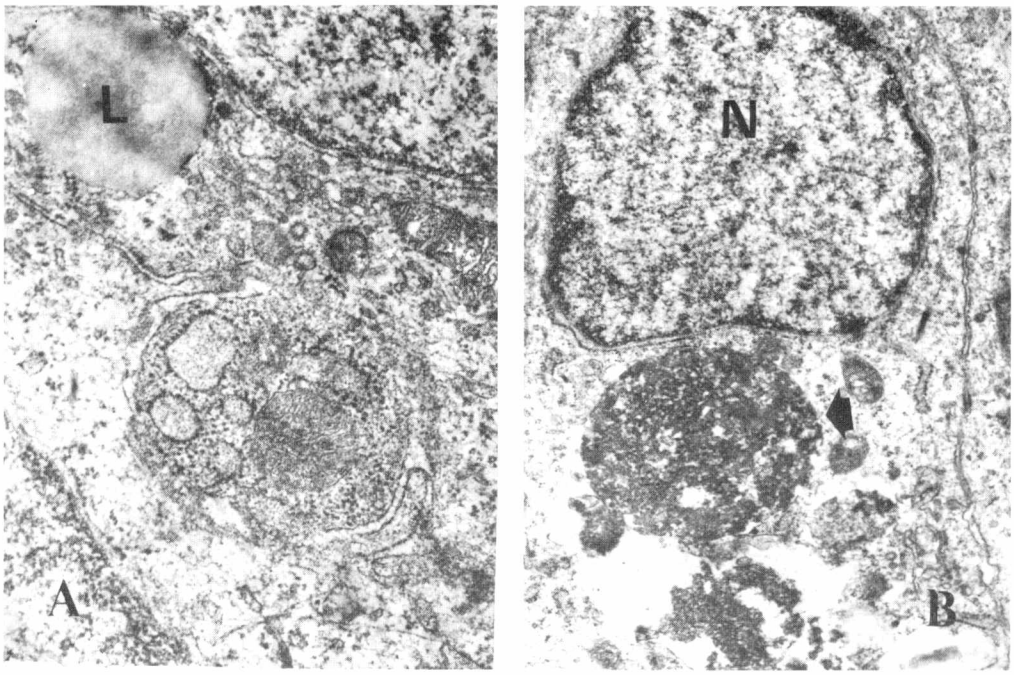

FIGURE 2 :
Lesions produced in an ameloblast 24 hs. post-irradiation.
A) A large lipid vacuole (L) in another cytoplasmic area (x 9,600).
B) An irregular intracytoplasmic granule (arrow) is seen in the vacinity of the nucleus (N).

Electron microscopy

At the ultrastructural level, ameloblasts showed some alterations as early as 24 hr after irradiation. Large lipid vacuoles and irregular intracytoplasmic secretory granules, four or five times larger than those observed in the controls, were frequently seen **(Fig. 2).** In two experiments of the eight-day group, a depolarization of the basal group of mitochondria was detected.

The degree of cell damage varied greatly between cells within the same section. Greatly altered cells alternated with ameloblasts in which an increase of lipid vacuoles was the only visible change.

The odontoblast layer evidenced multiple regressive alterations. Cytoplasmic vacuoles and numerous myelin figures were the most common features observed. Most cells suffered a reduction in size and an increase in election density **(Fig. 3).**

No evidence of change in the nuclear polarity was noted.

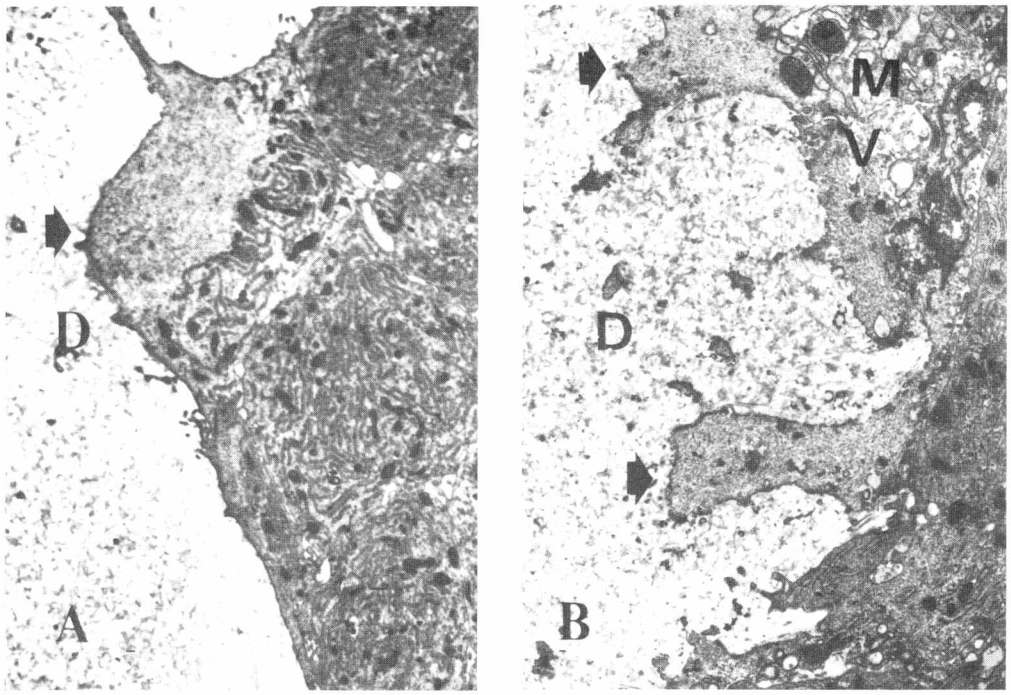

FIGURE 3 :
Odontoblastic area, 5 days after irradiation.
A) Increased cytoplasmic density and irregularly conformed odontoblastic processes surrouded by predentin (D).
B) Predentin (D), abnormal odontoblastic processes (arrows) and myelin figures (M) and vacuoles (V) in the cytoplasm. (x 5,800).

Numerous mesenchymal pulpal cells showed signs of active secretory activity. These cells had hyperplastic endoplasmic reticulum and had developed odontoblastic processes. These odontoblastic processes, although irregular in shape, exhibited the characteristic light staining and microfilament and microtubule content. In some instances, two or more processes developed at opposite sites of the same cell. The active mesenchymal cells were surrounded by a fibrillar matrix similar in appearance to the predentin matrix formed by the odontoblasts before the irradiation **(Figs 4 and 5).**

Whereas most mesenchymal cells showed active changes, a few of them exhibited regressive changes similar to those observed in the odontoblastic layer and became enclosed in the osteodentin matrix.

DISCUSSION

The degree of radiosensitivity of the different cells in the developing tooth has been discussed by several authors. It has been said that amelogenesis is more radiosensitive than odontogenesis (English and Tullis, 1951 ; Dale,

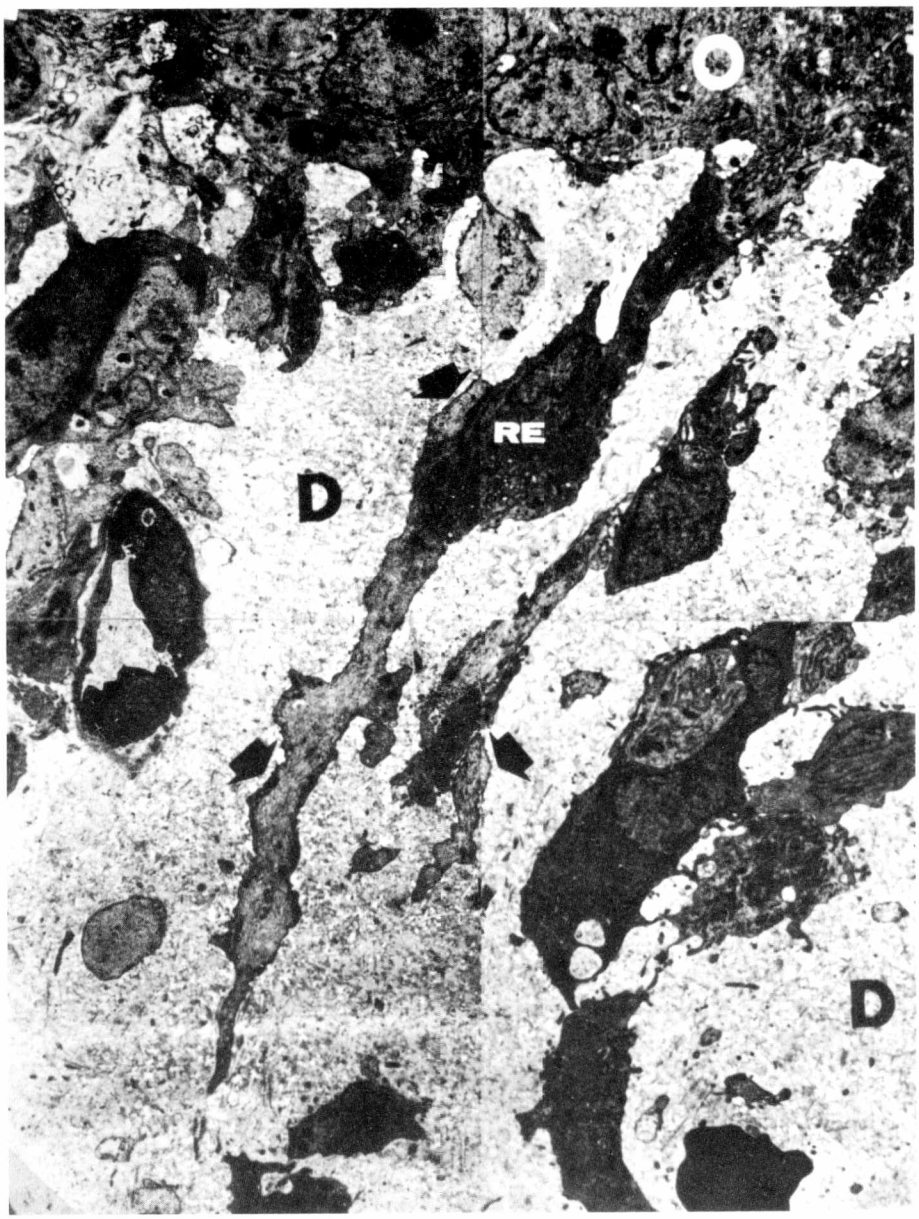

FIGURE 4 :
Developing molar tooth, 3 days after irradiation. Mesenchymal pulpal cells with hyperplastic endoplasmic reticulum (RE) and irregular odontoblastic process formation (arrows) surrounded by dentin matrix (D). O : odontoblastic layer. (x 3,200).

FIGURE 5 :
General pattern of odontongenesis area in a normal (A) and an irradiated tooth germ (B). 8 days post-irradiation, the odontoblastic layer (O) became trapped between the predentin (P) and the internal formation of osteodentin (IO) produced by pulpal mesenchymal cells (M) with odontoblastic differentiation. (x 1,600).

1953 ; Lindvall et al., 1972) and the well differentiated odontoblasts are relatively radiosistant (Medak et al., 1952 ; Pliess and Franke, 1960 ; Collet et al., 1966 ; Adkins, 1967 ; Koppang, 1973). However, it seems to be clearly proved that radiosensitivity varies according to the different stages of differentiation and function of tooth germ cells.

In the developing molar teeth used in the present study, most ameloblasts showed distinct ultrastructural alterations. However, more severe damage occurred in the odontoblasts, which showed a depressed secretory function and became trapped between the dentin formed before irradiation and the osteodentin produced by the stimulated pulpal cells. This observation indicates that the observed alterations in the odontoblasts might be a consequence of a deficient nutrition rather than of a direct damage produced by irradiation.

Pulpal mesenchymal cells exhibited a particular pattern of behavior. The most striking feature was the absence of cytoplasmic breakdown and rapid odontoblastic differentiation with dentin matrix secretion. This stimulation of pulpal cells appears to be similar to that observed in pulpal wound repair (Fitzgerald, 1979) ; and in formation of secondary dentin and pulp stones.

Different degrees of differentiation capacity were observed among pulpal cells. Osteodentin production always begun at the level of the initiation of Hertwig's sheeth, in a zone of active proliferation. This observation is consistent with the findings of Burton (1949), who reported a distinct alteration of root formation after irradiation of mouse developing teeth with doses of 1,500 to 5,000 r.

Even with the high dose of 16,000 r used in the present study, the mesenchymal pulpal cells, unlike other undifferentiated cells, did not undergo regressive changes. On the contrary, irradiation stimulated a high potential for dentin production in these cells.

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