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An autoradiographic study of the response of rat molar pulp to formocresol using ^{3}H-thymidine

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Abstract

Molar pulps of rats were exposed and treated with formocresol and studied autoradiographically following the administration of ^{3}H-thymidine. A profound suppression of mitotic activity was observed in the coronal pulp one day following formocresol treatment. At three days a substantial number of labeled pluripotential cells were located above and below a necrotic zone of tissue. By one week this zone was infiltrated with viable, labeled cells, forming a cellular bridge which isolated the wound site. Elaboration of a dentin bridge within four weeks was accomplished, without further mitosis, by odontoblasts derived from precursor cells. This study demonstrated a reversible influence on pulp cells beyond the zones of necrosis usually associated with formocresol.

The formocresol pulpotomy has become an accepted clinical procedure for the treatment of primary teeth showing signs and symptoms of pulp inflammation since it was first advocated by Sweet.[1] Since then no definitive theory has been advanced to explain the clinical efficacy of formocresol despite its dismal biologic attributes.[2]

Numerous studies have analyzed the histologic and enzymatic response of human and animal pulps to formocresol,[3-11] comprehensive reviews describing these findings have been published in recent years.[2, 12] Further insight into the mechanisms of action has been gained from biochemical studies on formocresol-treated connective and pulp tissue.[13-15]

Efforts have been made to determine the effects of cresol and formaldehyde—the active components of formocresol[9]—and to determine the fate of formaldehyde following the introduction of formocresol into the pulp.[11, 16, 17]

Various investigators have used ^{3}H-thymidine to investigate the response of rat molar pulps to a variety of insults.[18-23] This study was undertaken to gain additional knowledge about the response of pulp cells to a strong cytotoxic agent, formocresol, using ^{3}H-thymidine as a marker for mitosis.

Methods and Materials

Forty-two rats weighing 100 gms each were used in this study. In all procedures the animals were anesthetized with ether. The pulp of one upper maxillary first molar in each rat was exposed by a technique previously described,[9] the antimere tooth was left untouched to serve as a control. All operated teeth were treated for five minutes with a commercial preparation of formocresol[a] and covered with a zinc oxide and eugenol cement mixed with equal quantities of eugenol and formocresol. The cavity preparation was then sealed with amalgam.

Subsequent to the experimental procedure, the animals were sacrificed at the following time periods: three hours, one day, three days, one, two, three, and four weeks. One hour prior to sacrifice, ^{3}H-thymidine (Methyl-^{3}H, 6.7 Ci/mmol, New England Nuclear) at a dose of 1 μ Ci/gm body weight, was injected IP. The rats were perfused in vivo according to the technique of Jensen[24] using a glutaraldehyde solution as formulated by Rostgaard and Behnke.[25] After in vivo fixation the entire maxilla was separated from the head and placed in buffered EDTA for demineralization. The teeth were washed, dehydrated, embedded in paraffin and sectioned serially at 4 nm. Mesiodistal serial sections were placed on glass slides and coated with Ilford K5 autoradiographic emulsion. After a month of exposure, they were developed and then stained with methyl green-pyronine. Some sections not used for autoradiography were stained with hematoxylin and eosin.

Results

Three-Hour Specimens

The cells below the exposure were compressed somewhat by the restorative procedure but trauma was kept

[a]Buckley's Formocresol, Crosby Laboratories, Burbank, Calif.

to a minimum (Figure 1). Despite the minimal physical insult, no label could be observed in the entire coronal pulp chamber of the formocresol-treated specimens. Although the cells of the pulp exclusive of the apical region are acknowledged to be a very stable population,[23] occasional labeling of fibroblasts and endothelial cells always were seen in the control teeth (Figure 2). Both control and experimental teeth exhibited similar active cell division in the apical region, however (Figure 3).

One-Day Specimens

There was virtually no increase in mitosis throughout the entire coronal pulp of experimental teeth compared to the control teeth. The only change over the three-hour specimens was a slight infiltration of inflammatory cells.

Three-Day Specimens

The classic effects of formocresol were now in evidence (Figure 4). A compressed, deeply stained area of debris and fixed tissue was seen immediately beneath the exposure site. Next, a wider, pale-staining, edematous, and ill-defined zone blended into a third zone of viable pulp which was infiltrated by inflammatory cells (Figure 5). Two bands of mitotic cells were associated with the pale, intermediate zone. One layer of dividing cells (Figure 6a) was situated just under the exposure site and another (Figure 6b) ringed the amorphous zone distal to the opening. In the intermediate zone between these two bands of cell division, virtually no labeling could be seen.

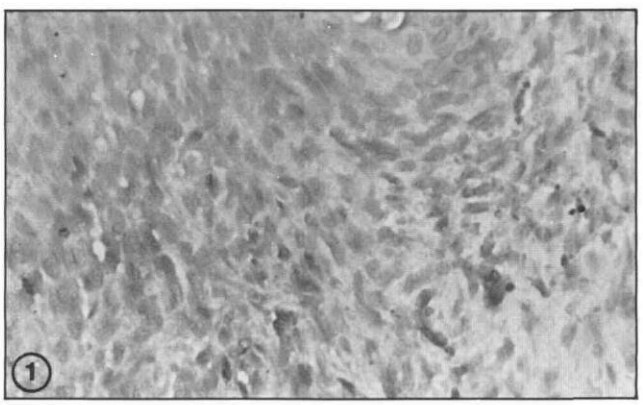

Figure 1. Three-hour specimen shows the compressed cells below the exposure; no mitotic activity is seen in the coronal region (Original magnification 720x).

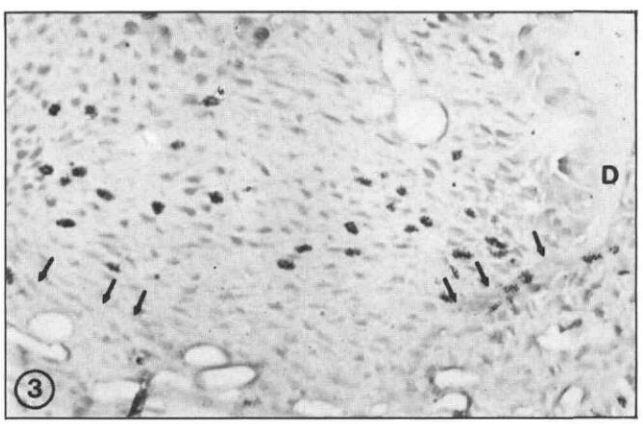

Figure 3. Control specimen, showing active cell division in the apical region. (D denotes root dentin and arrows point to Hertwig's epithelial root sheath, original magnification 450x).

One-Week Specimens

By one week, the pale-staining amorphous zone had been replaced by a barrier or bridge of cells, separating the wound site from the normal pulp. Interspersed throughout this new formation were labeled cells, suggesting that the former amorphous zone was infiltrated and replaced by actively dividing cells (Figure 7).

Two-Week Specimens

The cellular barrier of one week had matured toward a primary bridge, exhibiting condensation of cells, dep-

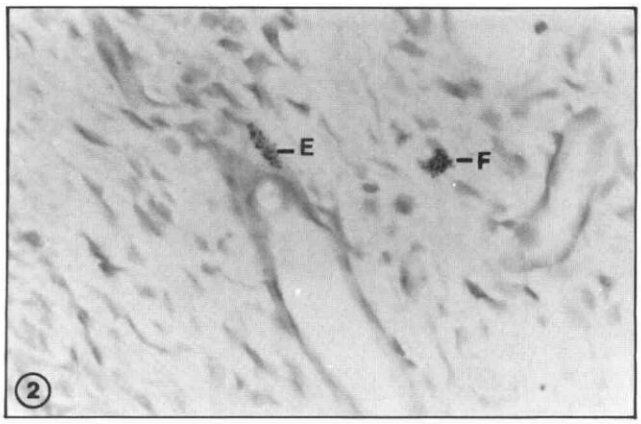

Figure 2. Mitotic activity in the coronal pulp of a control specimen, demonstrating the slow turnover of cells in the coronal pulp (E designates endothelial cell or pericyte and F a fibroblast, original magnification 720x).

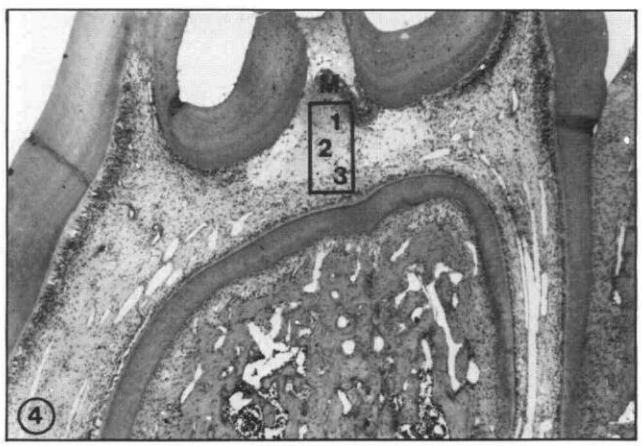

Figure 4. Three-day specimens, showing medicament (M) and the classic zone of formocresol (insert): fixed tissue (1), pale-staining, edematous zone (2), and viable pulp infiltrated by inflammatory cells (3) (Original magnification 60x).

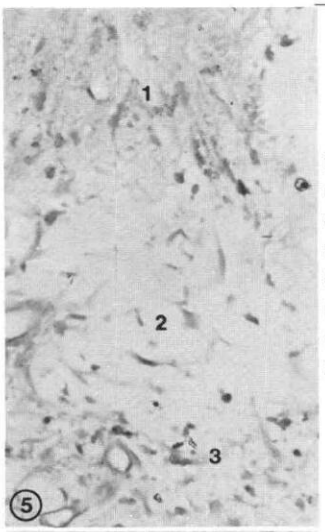

Figure 5. Enlarged view of the characteristic zones of formocresol: band of mitotic cells just under the exposure site (1), an intermediate amorphous zone (2), and the region distal to the amorphous zone (3) (Original magnification 450x).

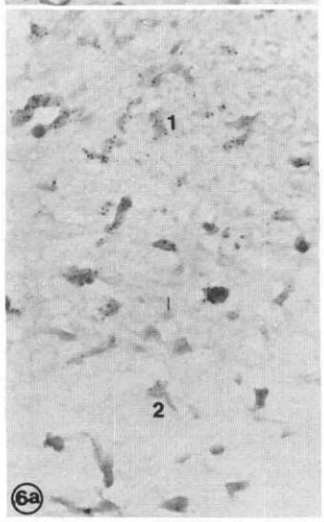

Figure 6a. Enlargement of areas one and two in Figure 5 showing layer of dividing cells (1) and part of amorphous zone (2). (Original magnification 720x).

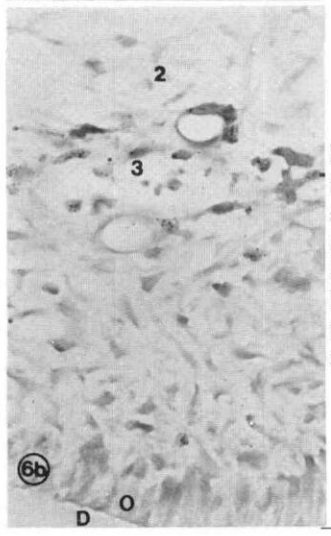

Figure 6b. Enlargement of areas two and three in Figure 5, showing the amorphous zone (2) and a band of mitotic cells (3). Also shown are odontoblasts at the floor of coronal pulp and dentin (D). (Original magnification 720x).

osition of matrix, and the appearance of odontoblast-like cells. Bands of mitotic activity associated with the repair process had disappeared by this time, and labeling was infrequent and random, similar to the control teeth.

Three- and Four-Week Specimens

Reparative dentin bridges were formed as the earlier cellular proliferations were consolidated (Figure 8). No extraordinary mitosis was associated with this stage as

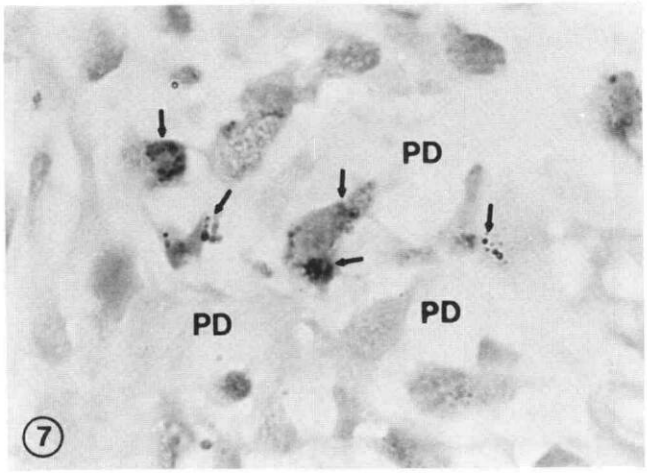

Figure 7. One-week specimen demonstrating the cellular bridge containing labeled cells (arrows) and predentin (PD). (Original magnification 1800x).

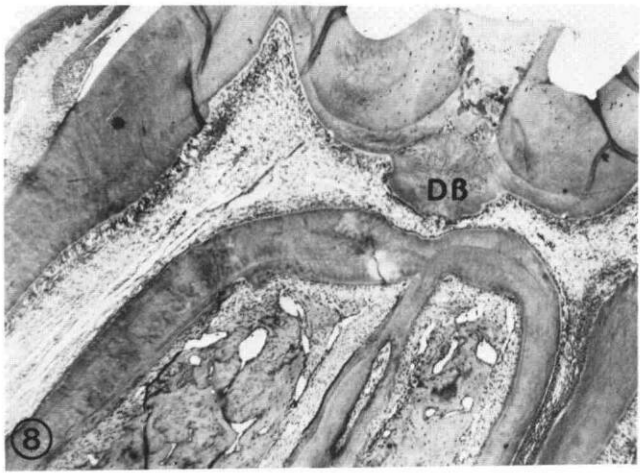

Figure 8. Four-week specimen, demonstrating a successful reparative dentin bridge (DB). (Original magnification 60x).

labeling resembled that of control teeth. Apical cell division was still a prominent feature after four weeks.

Discussion

The most active period of cell mitosis occurred between the third day and one week following the subjection of the pulp to formocresol. Prior to this time, at three hours and one day, there was virtually no evidence of mitosis throughout the entire coronal pulp. This inhibition of cell division was manifested beyond the area of compressed cells, where physical changes alone could explain the results.

Although there is a low turnover of cells in normal pulps,[23] a finding verified by our control teeth, there is no doubt that the procedure dramatically suppressed division of cells. This inhibition extended beyond what is considered to be the zone of fixation or cell death, and suggests that diffusion of formaldehyde through the pulp can influence cell metabolism without actually

killing the cells. This hypothesis is supported by the demonstration that the respiration of rat pulps is depressed after exposure to formocresol.[26] In contrast, a study by Sveen and Hawes[22] showed an increase in mitotic activity by 24 hours where cuspal grinding served as an irritation to the pulp and no medicaments were used.

By three days the multizones typically seen beneath formocresol began to develop. The pale-staining zone with loss of cellular definition had appeared, sandwiched between the condensed layer coronally and the inflammatory zone apically. This pale zone probably represented a combination of cell membrane destruction by the lipophilic agent cresol, cell necrosis from vascular stasis, and edema. Two zones of mitosis were seen associated with this pale zone, one immediately subjacent to the fixed, compressed cells under the medicament, and another beneath the pale zone in the region normally associated with inflammation. The origin of the cells closest to the exposure site remains undetermined by this experiment. However, because the cytotoxicity of formocresol is so well acknowledged, it seems inconceivable that cells in the immediate vicinity of the exposure could have survived. Instead, it seems reasonable that cells, probably fibroblasts, migrated through the pale zone to an area where the walling-off process could begin. By virtue of the timing of the ^{3}H-thymidine injection, it is certain that some of these cells underwent mitosis after moving into the injured area. Very few mitotic cells were seen within the pale zone; apparently, the process of division began only after migration was complete.

Cell division and migration continued unabated, because by one week the pale zone had been replaced by a cellular bridge which separated the site of insult from the pulp below. At this point the mitotic activity was interspersed throughout the cells of this bridge (Figure 6).

Subsequent to the one-week time period the repair process followed the typical pattern seen in rat molars, displaying a condensation of the cellular bridge and a deposition of matrix.[9] Odontoblasts began to differentiate and to lay down the reparative dentin. The mitotic activity during this phase was very low, indicating that the odontoblasts were derived from precursor cells, and these in turn were regenerated within a week or so following the exposure.

After four weeks the repair process matured, but mitotic activity had dropped to the level of the control teeth. It is obvious that little cell division is required after the first week in the process of forming a dentin bridge across a wound site. Healing of a pulp exposure in rats is a typical response, even to formocresol, provided the opening is sealed from the oral environment.[7, 9] The authors are unaware of any studies reporting similar responses in mammals, and thus it should not be interpreted that formocresol can stimulate reparative dentin like $Ca(OH)_2$.

This study verified the findings of others; that the pulp normally is composed of a very stable population of cells and that odontoblasts are postmitotic cells regenerated by modulation from more primitive precursors. Also disclosed is the capacity of the pulp to rebound from the initial shock of formocresol with active mitosis and migration of cells to seal off the irritant. Although no such response has been noted or can be expected in human primary teeth, this study suggests that cells are capable of recovering from the medicament.

Earlier studies by Loos et al.[27] demonstrated a speedier recovery of connective tissue cells exposed to dilute formocresol compared to those subjected to the full-strength medicament. With this in mind, preservation of the maximum number of viable pulp cells through the dilution of formocresol merits consideration as a clinical objective.[28] However, in light of recent concern about the possible carcinogenicity and/or mutagenicity of formaldehyde,[29] adoption of an alternative drug such as glutaraldehyde[30] might be a more responsible modification of the pulpotomy technique.

Summary

For a short period following its application, formocresol suppressed mitotic activity in the pulp beyond the zone normally associated with fixation or cell death. Within three days mesenchymal cells were dividing and migrating into the necrotic zone to begin the process of isolating the wound site. After one week a cellular bridge spread across the exposure and considerable mitosis occurred within it. By two weeks the cellular bridge had condensed, showing early dentin matrix formation, but the level of cell divisions had already returned to control levels. The healing process at three and four weeks was also accomplished without further elevated levels of mitotic activity. This study demonstrated that the pulp cells rebounded from the effects of formocresol with active mitosis within the first week, followed by migration and differentiation during the remainder of the healing process.

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Quotable Quotes

Izaak Wirszup of the University of Chicago has compared the effectiveness of the science-education systems in the United States and the USSR. For many years Russian schools offered an excellent education for a technical elite but little education for the masses. The system was reformed in 1966, however, and today 98% of all students in the USSR complete secondary education, compared with 80% in the United States. Russian students are required to take 10 years of geometry, 5 years of physics, 6 years of biology and 4 years of chemistry, compared with 1 year of each subject in most American schools.

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