Pulp reactions to exposure after experimental crown fractures or grinding in adult monkeys

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The purpose of the investigation was to assess the depth of inflammatory reactions in the exposed pulps of crown-fractured and ground teeth. Thirty-six incisors in 11 adult monkeys were used for the experiments. In 24 teeth, the pulp was exposed by grinding or by crown fracture, and the pulpal reactions were examined after 3, 48, and 168 hours. In three-hour specimens, the depth of early changes, namely hemorrhage and damage to the odontoblastic layer, did not exceed 2 mm from the exposure surface. The depth of inflammatory changes in 48-hour specimens ranged from 1.5 to 2.0 mm (mean 1.8 mm), and in 168-hour specimens from 0.8 to 2.2 mm (mean 1.6 mm). Among pulps exposed for 48 and 168 hours, 13 showed hyperplastic reactions in the exposure; in two teeth, the exposed pulp was only covered with fibrin, whereas the pulp in one tooth was necrotic. In the remaining 12 teeth that were used as a comparison group, the pulp was exposed by preparing a cavity, which was left open for 48 and 168 hours. The depth of inflammatory changes in the pulps ranged in 48-hour specimens from 1.0 to 9.3 mm (mean 3.8 mm) and in 168-hour specimens from 1.0 to 8.2 mm (mean 4.4 mm). In comparison with ground or crown-fractured teeth, inflammatory changes in the pulps exposed by cavity preparation were significantly increased ($P = 0.01$).

In a previous study,[1] patients with crown-fractured incisors, whose ages ranged from 7 to 16 years, had a high frequency of pulpal healing when the exposed pulp had been treated with partial pulpotomy, that is, when approximately 2 mm of the superficial layers of the pulp beneath the exposure was removed and the wound was treated with calcium hydroxide. The 2-mm depth of amputation was determined by the requirement that the cavity should be able to hold both dressing and sealing material, and, by the assumption that inflammatory changes in the pulps of recently injured teeth or in injured teeth showing hyperplastic tissue in the exposure were likely to be superficial. This latter assumption appears to have been confirmed by a recent study[2] in which the pulps in immature teeth of young monkeys were exposed for 4, 48, and 168 hours by grinding off a part of the crown. Among the pulps exposed for 48 and 168 hours, 17 of 22 showed hyperplastic reactions, which were invariably associated with either no inflammation or only superficial inflammation. However, the depth of inflammation was not specified. It is questionable to assume that the reactions in the ground teeth would be similar to those in crown-fractured teeth. Furthermore, findings that show an inferior response to injury in pulps of old rats compared with young rats[3,4] suggest that those results might have been less favorable had mature teeth of adult monkeys been included.

There are several reasons that make it important to determine the depth of pulpal inflammation in crown-fractured teeth to demarcate an optimal level for pulpal amputation. Calcium

hydroxide, commonly regarded as the preferred pulp dressing, should be placed in contact with noninflamed tissue, as it has no beneficial effect on the healing of inflamed pulp.[5] However, during pulpotomy, no tissue should be unnecessarily removed. Preservation of pulp tissue may have several benefits. By preserving the coronal cell-rich pulp tissue and reducing surgical injury the prospect of pulpal healing increases, especially if a cooled diamond and high-speed are used for cutting.[6] Sensitivity testing is also an option, which is lost if the whole coronal pulp is removed. Furthermore, physiologic dentin apposition may continue, especially in the cervical area of immature teeth, thereby reducing the risk of a later foot fracture.[7,8]

Proliferation of the pulp in crown-fractured teeth may be encouraged with the free exposure of the pulp, which permits continuous salivary rinsing and does not involve impaction of contaminated food debris.[1] Because no investigation has been conducted regarding this aspect, this study was designed to compare reactions in the pulps of crown-fractured teeth and pulps exposed by cavity preparation and left open to the oral environment. The purpose of this current paper was, therefore, to study the tissue changes in adult monkey pulps exposed by several methods with particular reference to the depth of inflammatory reactions at various time intervals.

MATERIALS AND METHODS

Forty incisor teeth from 11 vervet monkeys *(Cercopithecus aethiops)* were used for the experiments. All teeth showed complete root development on preoperative intraoral radiographs. Errors in histologic preparation made it necessary to exclude four teeth from the study. The remaining 36 teeth, which were 22 maxillary and 14 mandibular incisors, were evaluated.

Eight pulps were exposed by grinding off a part of the crown with a water-cooled diamond instrument in an air turbine. In 16 teeth, a groove was prepared in the enamel approximately 2 mm from the gingival margin, and the pulp was exposed by fracturing the crown. In 11 of these teeth, the crown was fractured with forceps, whereas in five, the crown was fractured by a blow from a hammer on a chisel that was placed into a prepared groove (Fig 1). In the remaining 12 teeth, the pulp was exposed by preparation of a cavity through the lingual surface of the crown with a water-cooled diamond instrument in an air turbine. All exposed pulps were left open to the oral environment. Distribution of the material is shown in Table 1.

After 3, 48, and 168 hours, the monkeys were killed with an overdose of sodium pentobarbitone. Their heads were perfused with physiologic saline solution, followed by 10% neutral buffered formalin. The teeth were removed in a tissue block, decalcified in 10% EDTA at pH 6.8, embedded in celloidin-paraffin, and step-serially sectioned through the pulp in a longitudinal plane, that is, sections 5 μm thick were taken at 50-μm intervals, and stained with hematoxylin and eosin.

Evaluation of the material was performed on separate occasions by two groups with two examiners each. When discrepancies occurred, values indicating the most extensive changes were recorded.

The depth of pathologic changes in the pulp was measured with a light microscope at a magnification of ×100 using a graduated eyepiece. The start-

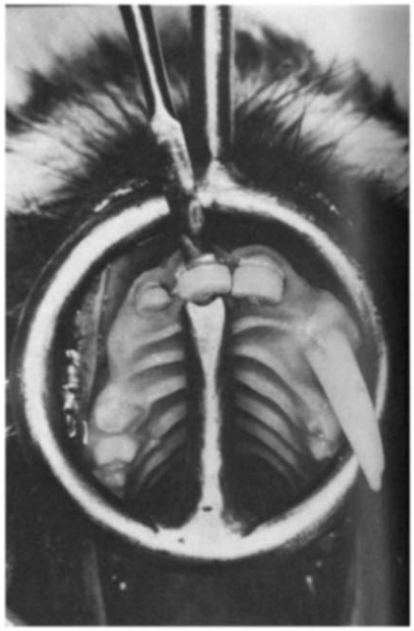

Fig 1—Maxillary right central incisor of adult monkey supported by instrument to prevent periodontal injury. Fracture of crown is performed by blow of hammer on chisel placed into prepared groove on labial surface of crown.

Table 1 • Distribution of the material according to the observation period and the type of pulp exposure.

Type of pulp exposure	Hours			
	3	48	168	Total
Grinding off a part of the crown	4	2	2	8
Fracture with chisel and hammer	4	†	1*	5
Fracture with forceps		6	5*	11
Cavity preparation		6	6	12
Total	8	14	14	36

*One tooth excluded because of error in histologic preparation.
†Two teeth excluded because of error in histologic preparation.

ing point was a line drawn across the pulpal lumen at the level of the lowest fracture angle of the pulpal exposure in an apical direction (Fig 2).

Damage to the odontoblastic layer was assessed if the odontoblasts were reduced in number or absent, or if they were displaced within the pulp or into the dentinal tubules. The degree of displacement of cells, either odontoblasts or leukocytes, into dentinal tubules was estimated from the percentage of involved tubules, that is, < 10%, 10% to 50%, and > 50%.[9]

The extent of inflammation was divided into the zone of infiltration and the zone of few, scattered inflammatory cells, and was measured at the level of the last extravasated inflammatory cell seen toward the apex of the tooth. The intensity of inflammation was judged from the density of inflammatory cell infiltration and was graded into slight, moderate, and severe, corresponding to grades 1, 2, and 3 according to Stanley's criteria.[10]

Hyperplastic tissue was assessed if fibroblasts and capillaries were present above the level of pulpal exposure. The height of the hyperplasia was defined as the highest point measured perpendicular to a line joining the two exposed angles (Fig 2).

In a statistical analysis of the results, a randomization test for independent values was used, and 5% was considered to be the critical probability level.[11]

RESULTS

The results are presented in Table 2. Because there were no statistical differences between ground and

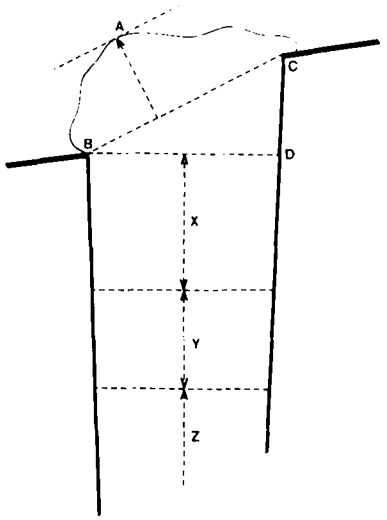

Fig 2—Levels from which histologic observations were measured. Height (A) of pulp tissue proliferation from line BC joining two exposure angles. From line BD, level of lowest fracture angle, depth of pathologic changes of pulp in root canal, which was divided into zone of infiltration (X), zone of few, scattered inflammatory cells (Y), and inflammation-free tissue (Z).

Table 2 • Mean value ($\bar{x}$) and range (r) of depth of measured variables 3, 48, and 168 hours after exposure of the pulp by grinding off a part of the crown or crown fracture in 23 teeth and, in 12 teeth, 48 and 168 hours after exposure by cavity preparation.

	Depth in millimeters (mm)						
			Inflammation:				
Ground and fractured teeth	Hemorrhage	Damage to the odontoblastic layer	Zone of infiltration	Zone of few, scattered cells	Total depth*	Total length of the pulp (mm)	Hyperplasia of the pulp (mm)
3 hours							
no. of teeth	8	8		2	2	8	
$\bar{x}$	1.0	1.2		0.2	0.2	10.5	
r	0.3-1.9	0.30–2.0	...	0.1-0.3	0.1-0.3	7.2-13.0	...
48 hours							
no. of teeth	8	8	8	8	8	8	6
$\bar{x}$	1.5	1.6	1.3	0.6	1.8	9.9	0.3
r	0.9-2.0	1.3-2.0	0.8-1.8	0.2-2.0	1.5-2.0	7.6-11.7	0.2-0.5
168 hours							
no. of teeth		7	7	7	7	7	7
$\bar{x}$		0.7	0.9	0.7	1.6	9.2	0.6
r		0.1-1.3	0.4-1.9	0.3-0.9	0.8-2.2	7.5-12.7	0.3-0.9
Cavity preparation							
48 hours							
no. of teeth	6	6	6	6	6	6	1
$\bar{x}$	1.9	0.8	1.3	2.6	3.9	10.4	0.2
r	1.3-3.0	0.3-1.1	0.4-2.5	0.5-7.5	1.0-9.3	8.2-14.0	0.2
168 hours							
no. of teeth		6	6	6	6	6	3
$\bar{x}$		1.6	1.6	3.3	4.4	8.7	0.2
r		0.8-2.7	0.7-3.0	0.6-7.3	1.0-8.2	5.0-12.0	0.1-0.2

*Total depth of inflammation = zone of infiltration + zone of few, scattered cells. The tooth showing necrosis of the pulp 168 hours after exposure by crown feature is not included in the table.

crown-fractured teeth in regard to pulp tissue reactions, the results were pooled.

Pulps exposed by grinding or crown fractures

Three hours after exposure

No proliferation of the pulp was seen. The surface of the exposed pulps consisted of damaged tissue covered with clotted blood. The changes in the pulps, which extended 2 mm into the pulp from the exposed surface, were characterized by hemorrhage and damage to the odontoblastic layer. The damage consisted of complete or partial loosening of odontoblasts from dentin, resulting in the formation of vacuoles along the dentinal wall (Fig 3) and displacement of odontoblasts into dentinal tubules. Nuclei of displaced cells were seen in 10% of tubules and at a short distance from the pulpal lumen.

Pavementing and polymorphonuclear leukocytes along the inner walls of dilated vessels was seen in three pulps, and, in two of these, a few such cells were observed outside the vessel walls.

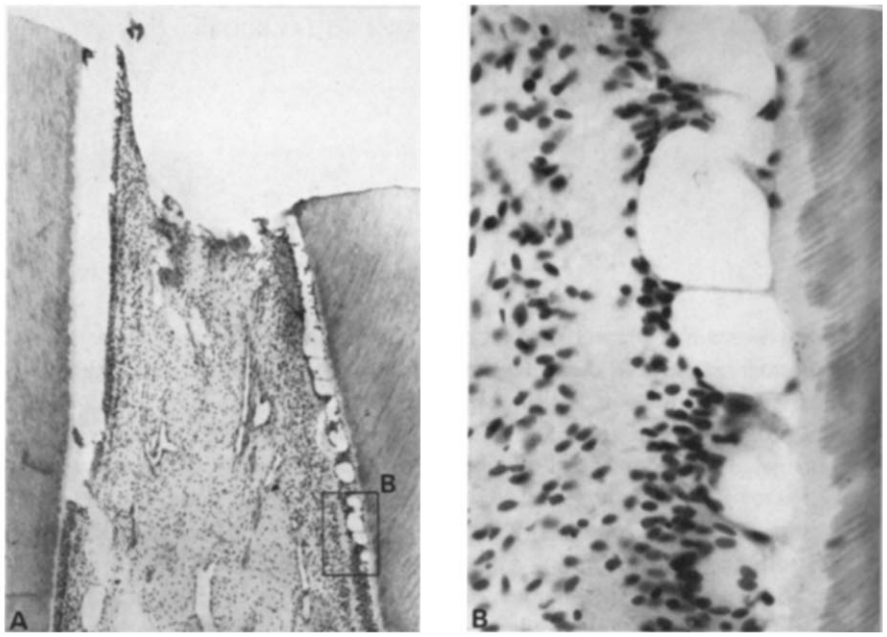

Fig 3—A, B, exposure three hours after grinding off part of crown. Debris and coagulated blood at surface; loosening of odontoblastic layer from dentin, complete at left and by formation of vacuoles on right, to depth of 1.3 mm (orig mag ×60 and ×200).

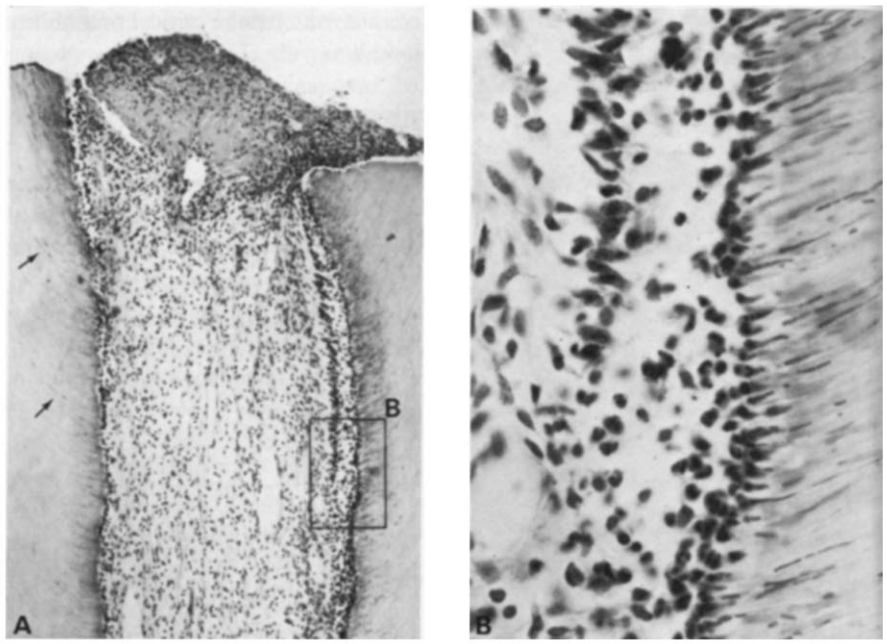

Fig 4—A, exposure 48 hours after fracture of crown. Exposure surface covered with fibrin and limited proliferation of pulp, rounding right angle of dentin; damage of odontoblastic layer and moderate infiltration with inflammatory cells to depth of 1.4 and 0.9 mm, respectively. Arrows point to displaced nuclei in dentinal tubules (orig mag ×90). B, cellular accumulation beneath damaged odontoblastic layer and displacement into dentinal tubules of polymorphonuclear leukocytes (orig mag ×300).

48 hours after exposure

Proliferation of the pulp tissue was seen in six teeth. In two of these, the pulpal surface was covered with some patches of fibrin, and proliferation of the pulp was limited to the periphery of the exposure (Fig 4, A). In the remaining two teeth, no hyperplastic response was observed, and the surface was entirely covered with fibrin.

The depth of inflammatory reactions, including the zone of few, scattered inflammatory cells, ranged from 1.5 to 2.0 mm (mean 1.8 mm). Infiltration, predominantly with polymorphonuclear leukocytes, was most defined along the dentinal walls within the zone of damaged odontoblastic layer (Fig 4, B). It was slight in two pulps, moderate in four, and severe in two. Nuclei of displaced cells, apparently leukocytes, occupied 10% to 50% of dentinal tubules in two teeth and more than 50% in six. These nuclei were seen in the tubules along the whole distance from pulpal lumen to the fracture surface.

168 hours after exposure

Proliferation of the pulp tissue through exposure was present in seven teeth, protruding at most 0.9 mm

above the exposure level (Fig 5, 6). In three teeth, proliferated tissue was partially covered with epithelium (Fig 5, B), and in two teeth, it contained nerve fibers (Fig 6, B).

The depth of inflammatory cell infiltration in the pulp ranged from 0.8 to 2.2 mm (mean 1.5 mm). Infiltration, with polymorphonuclear leukocytes and in lesser number, with lymphocytes, was slight in two teeth, moderate in three, and severe in two. Nuclei of displaced cells were seen in 10% of the tubules in five teeth and in 10% to 50% of the tubules in two. The nuclei were seen in tubules at all distances from pulpal lumen to the fracture surface. In one tooth, the whole pulp was necrotic.

In most teeth fractured with a forceps, dentin fragments were observed adjacent to the fracture surface. In two teeth, longitudinal fractures of the dentin were associated with severe infiltration of inflammatory cells in the adjacent pulp, whereas the opposite side of the pulp was free of inflammation. The presence of longitudinal fractures seems to have increased the depth of inflammatory reactions in these two teeth.

A comparison between the extent of inflammatory changes in the pulp after exposure of 48 hours and then after 168 hours showed no significant difference.

Pulps exposed by cavity preparation in the crown

48 and 168 hours after exposure

There were no differences between the pulps exposed for 48 and 168 hours. Cavities in the crowns of all teeth but one were filled with plaque and food debris often containing vegetable fibers. In most of the teeth, the

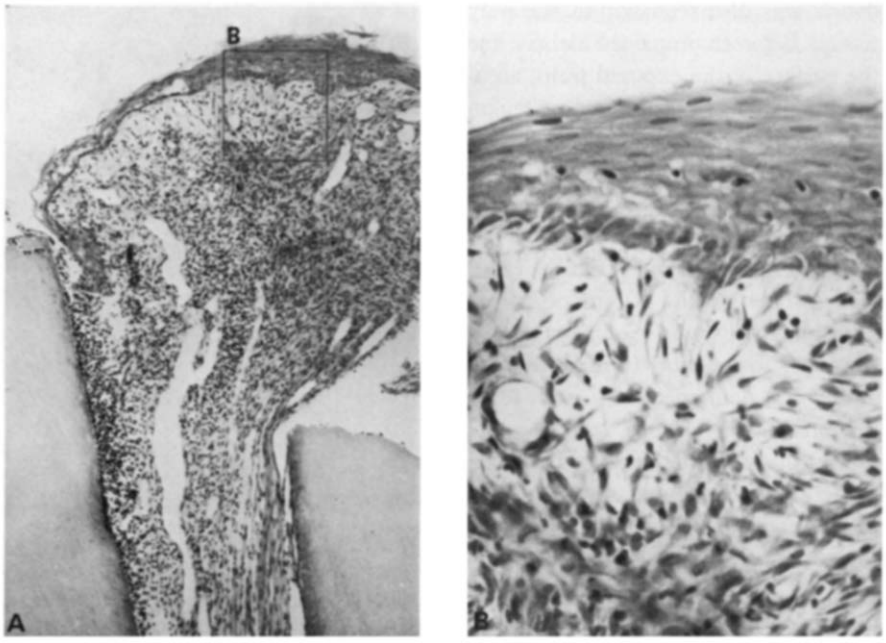

Fig 5—A, Exposure 168 hours after fracture of crown. Severe inflammatory cell infiltration of pulp in root canal to depth of 0.5 mm (orig mag ×70). B, epithelium covering surface of proliferated pulp tissue (orig mag ×200).

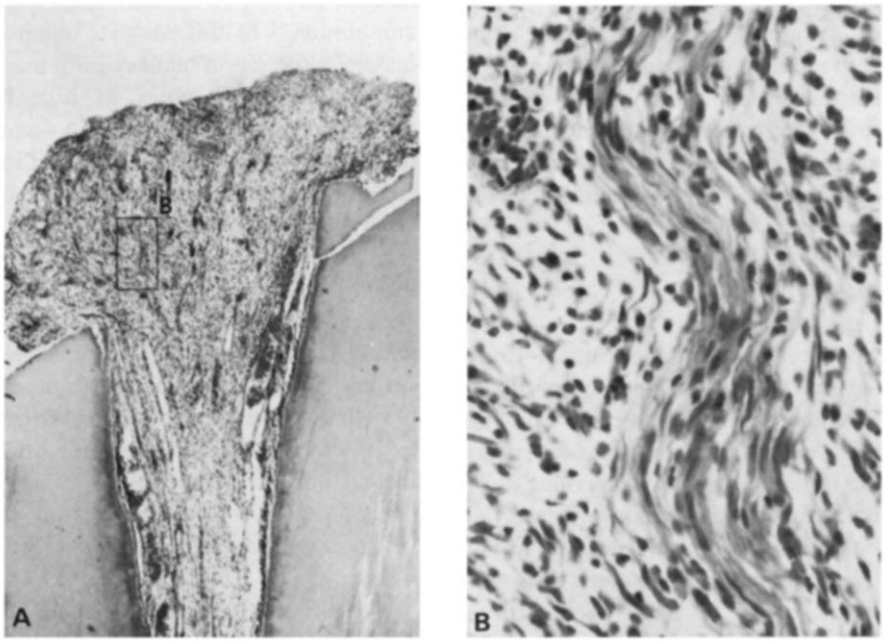

Fig 6—A, exposure 168 hours after fracture of crown. Moderate inflammatory cell infiltration of proliferated tissue and slight infiltration of pulp in root canal to depth of 0.4 mm (orig mag ×32). B, nerve fibers in proliferated pulp tissue (orig mag ×320).

debris was also seen within the pulp tissue. Between impacted debris and the surface of the exposed pulp, accumulation of polymorphonuclear leukocytes and faintly stained material were present in all teeth, indicating abscess and pus formation (Fig 7, A). Limited proliferation of granulation tissue was observed along the cavity walls in four teeth. The depth of inflammatory reactions in 48-hour specimens ranged from 1.0 to 9.3 mm (mean 3.8 mm) and in 168-hour specimens from 1.0 to 8.2 mm (mean 4.3 mm). Infiltration of the pulp with inflammatory cells was moderate in three, and severe in nine pulps (Fig 7, B). Displacement of cells into dentinal tubules was more pronounced in the 48-hour specimens than in the 168-hour specimens.

Comparing the extent of inflammatory changes in the pulp after 48 hours and changes in pulp after 168 hours of exposure showed no statistically significant difference in the cavity preparation group, whereas a comparison between the pulps in ground or crown-fractured teeth and those exposed by cavity preparation showed that the depth of inflammatory reactions in the latter group had significantly increased ($P = 0.01$, Table 2).

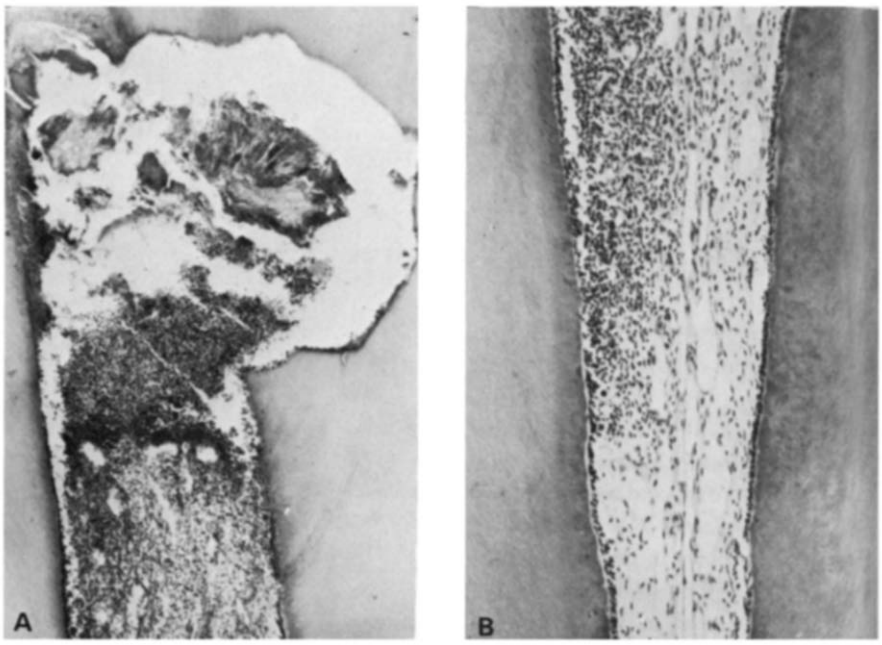

Fig 7—A, exposure 168 hours after cavity preparation. Accumulation of plaque and food debris, and abscess formation in the cavity (orig mag ×32). B, severe infiltration of pulp in root canal with inflammatory cells to depth of 3.0 mm (orig mag ×90).

DISCUSSION

Observations in the current study agree with earlier findings of tissue reactions in pulps exposed by grinding off a part of the crown[2] and support the results reported in a previous study on frequency of healing after partial pulpotomy in crown-fractured teeth.[1] Changes in the pulp exposed by grinding or crown fractures were surprisingly uniform, both in regard to the depth and to the character of inflammatory reactions. Age of animals and maturity of teeth appear to be of negligible or, possibly, of only minor significance as similar tissue reactions were found in immature monkey teeth,[2] as well as in multirooted teeth of aged patients two weeks after root amputation.[12] In this context, inflammatory responses of monkey pulp may be similar to responses of human teeth.[13] Thus, the current results seem to warrant the recommendation that in crown-fractured teeth that show vital or hyperplastic tissue in the pulp exposure, or both (a common finding in accidentally injured incisors[1]), surgical incision should be made approximately 2 mm beneath the exposure surface. This provides a safe amputation level considering the average extent of inflammation observed within the time investigated. The experimental period corresponds to that in which most patients come for treatment.

No difference could be found in pulp tissue reactions between the pulps exposed by grinding and pulps exposed by crown fracture. Fracture with forceps, however, caused crushing and longitudinal fractures of dentin (Fig 8). Though such damage may also occur in accidentally injured teeth, using forceps for experimental fractures appears less suitable compared with using chisel and hammer or grinding off a part of the crown.

Fig 8—Exposure 48 hours after fracture of crown with forceps. Multiple fractures of dentin (orig mag ×15).

Exposure of the pulp by cavity preparation resulted in impaction of food debris and abscess formation in the cavities of all teeth. This finding and comparison with ground and crown-fractured teeth support the assumption that a free exposure, which permits salivary rinsing and rules out impaction of contaminated debris, encourages a proliferative response in exposed pulp.

CONCLUSION

There are two essential objectives for surgical treatment of the vital pulp that make the level of pulpal amputation important: a wound dressing should be placed on the noninflamed tissue, and the loss of tooth substance should be kept to a minimum. From the results of the current study, in which the depth of inflammatory changes in exposed pulps of monkey incisors was assessed, the following conclusion can be drawn for clinical use. In crown-fractured teeth, showing vital pulp tissue after an exposure period of up to seven days after injury, not more than 2 mm of the pulp beneath the exposure needs to be removed, that is a partial pulpotomy can be performed for the effect of calcium hydroxide to be exerted on noninflamed tissue. Partial pulpotomy causes only limited injury to the pulp and limited loss of tooth substances, which is important for pulpal healing and facilitates subsequent restoration of a fractured crown.

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