Revitalization of pulpless open apex teeth in rhesus monkeys, using collagen-calcium phosphate gel

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Apexification has been achieved in pulpless open apex teeth in monkeys by means of a collagen-calcium phosphate gel. The process appears to be one of revitalization because the connective tissue ingrowth appears in various forms, including bone.

When the pulp tissue of a developing or a mature tooth is damaged by trauma or caries, root canal therapy must be initiated. Various obturation techniques including the use of cements, silver cones, and gutta-percha have been used in past years with some degree of success. However, the physical properties and cytotoxicity of these materials have limited endodontics to an art form that is costly to the patient and difficult to reproduce.[1] These problems are particularly evident concerning the pulpless open apex tooth. Thin dentinal walls and flaring apical morphological characteristics make routine obturation techniques extremely difficult. Often, root canal cements and gutta-percha are forced through the open apex, causing inflammation of the periapical tissues, resorption, and ultimate failure.

Recently, an apexification technique using calcium hydroxide paste has been advocated in an attempt to induce hard-tissue bridging.[2] However, hard-tissue closure usually requires from 9 to 18 months to form, and the result is most often a thin, porous calcific bridging limited to the apical portion of the root canal.[3] Final obturation of the canal with gutta-percha is usually necessary.

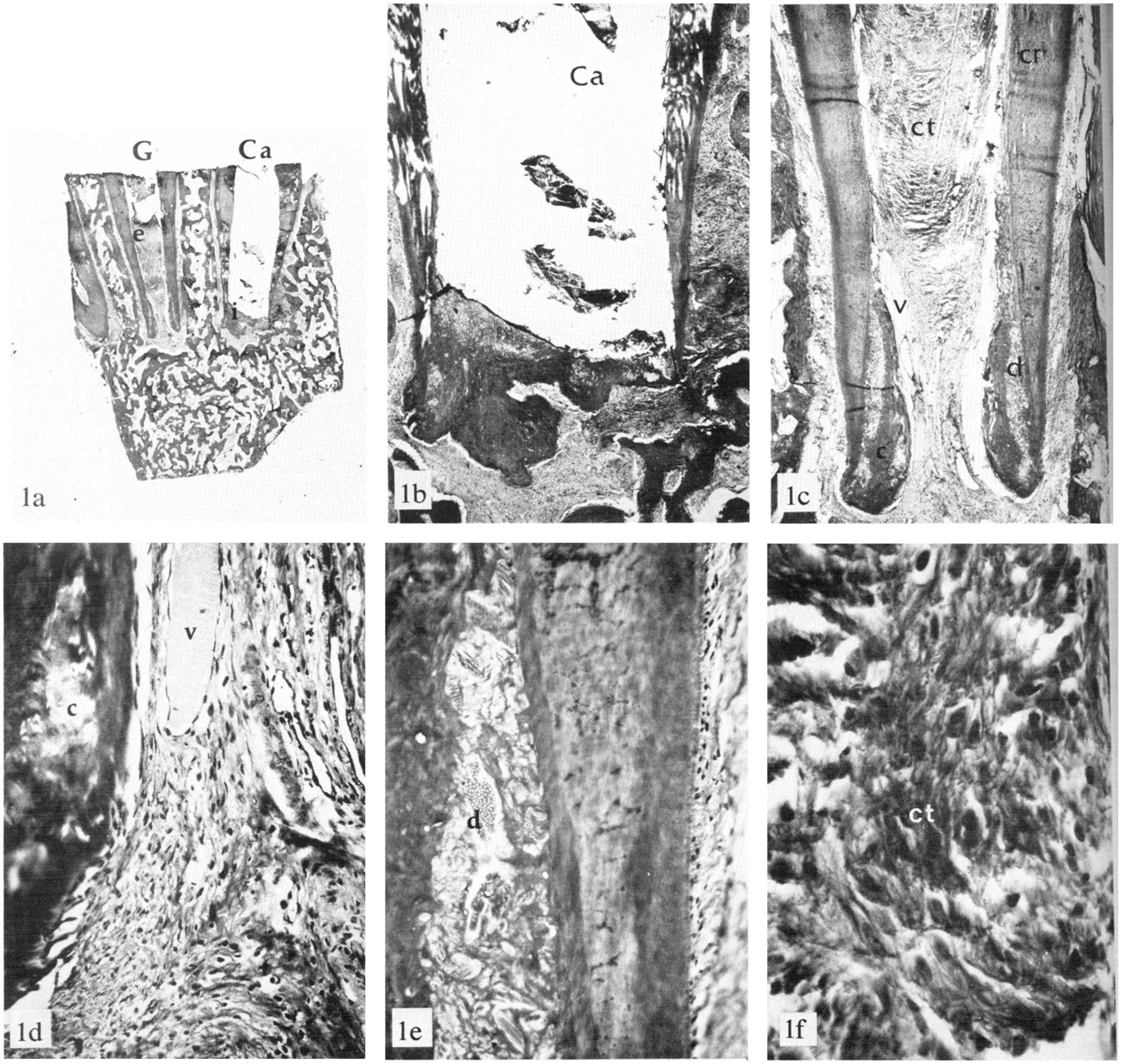
In an experimental approach to solving this problem, decalcified allogenic bone matrix grafts were surgically implanted into periapical tissues of teeth in rhesus monkeys.[4] Formation of new cementum at the apices and bone within the surgically formed bone cavities was observed. This technique has the following drawbacks: a surgical procedure is required; it requires the use of a nonpurified material; and the implant does not conform to the shape of the canal.

More recently, it has been shown that mixtures of collagen-calcium phosphate gel induce physiologic closure of subcutaneous polyethylene tube implants.[5] The openings of several tubes that had been filled with the gel were occluded by dense scars of mineralized connective tissue. Differentiation of mesenchymal cells to palisading fibroblastlike cells and elaboration of a linear collagen matrix at the tissue-gel interface were evident.

The present study was conducted to determine if collagen-calcium phosphate gel would induce physiologic closure of biomechanically debrided open apex teeth in rhesus monkeys.

Materials and Methods

Calfskin collagen* was reconstituted (10 mg/ml) in a 0.1-M acetate buffer, at a pH of 3 to 5 at 4 C to produce a viscous gel. This then was dialyzed against a 0.115-M phosphate buffer at a pH of 7.6 at 4 C for 24 hours.



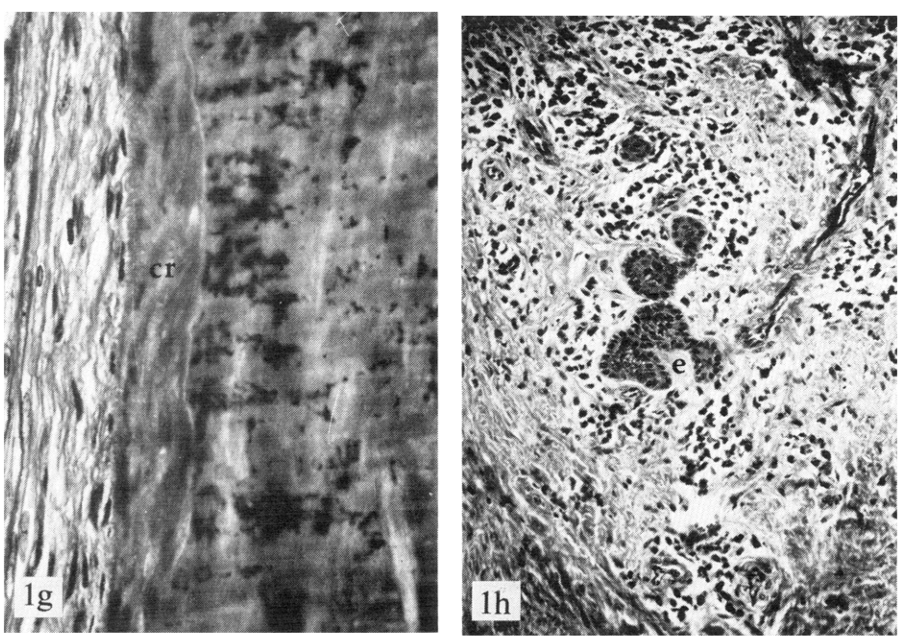


Fig 1a—G, mandibular incisor treated with collagen-calcium phosphate gel, showing ingrowth of connective tissue; e, location of epithelium adjacent to gutta-percha seal; Ca, adjacent incisor treated with calcium hydroxide-normal saline paste; i, incomplete apical bridge of hard tissue (Mallory's trichrome, orig mag × 10); 1b—detail of Ca (Mallory's trichrome, orig mag × 50); 1c—detail of mandibular incisor: c, cementum deposition constricting apical architecture; d, dentin chips encased in cementum; v, blood vessel; ct, fibrous connective tissue; cr, cementum repair of instrumented root canal wall (Mallory's trichrome, orig mag × 50); 1d—higher magnification of

Solutions of calcium chloride ($CaCl_2$ 2.0 M) and dipotassium hydrogen phosphate (K_2HPO_4 1.8 M) were prepared in Tris HCl buffer† (pH 7.4, ionic strength =0.15) and the pH adjusted back to 7.4. Small quantities of these salt preparations were added to the collagen gel solution to serve as calcium and phosphate ion sources.

Maxillary and mandibular central incisor teeth of four rhesus monkeys about 2½ years of age, were selected as experimental models for open apex teeth. All teeth were debrided biomechanically and irrigated with normal saline solution.

In two of the animals (group A) all incisors were left open to salivary contaminants for a period of one week. These teeth were then reinstrumented, irrigated with saline, and closed with sterile cotton and IRM.‡ One week later all teeth were reopened, cultured by use of tryptic soy broth with 0.1% agar,§ and reirrigated. All cultures subsequently were found to be positive at 72 hours. The teeth were filled at this time with the gel material. A control tooth in each animal was filled with calcium hydroxide-normal saline paste. Coronal seal consisted of short prefitted gutta-percha cones and IRM.

In two other animals (group B), a one-visit procedure was done in which incisor teeth were debrided, irrigated with normal saline, and filled with the gel material. No cultures were taken. A control tooth in one animal was filled to the radiographic apex inadvertently with a gutta-percha cone and no endodontic cement. A control tooth in the other animal was left empty and sealed coronally with IRM.

Radiographs of all teeth were taken at 4, 8, and 12 weeks. Blood samples also were drawn at these time intervals and Ouchterlony and ring or interfacial tests were conducted to determine antibody formation to the collagen gel.[6]

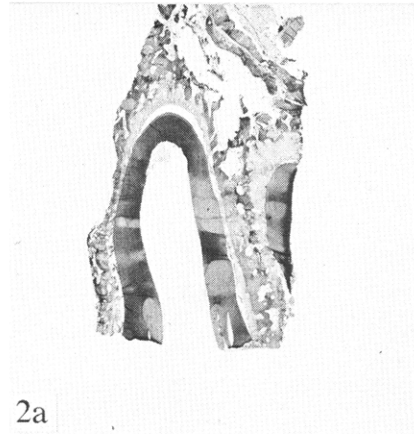
All animals were killed at 12 weeks, and block sections containing the teeth were excised. Bone sections were fixed in 10% neutral buffered Formalin and decalcified in RDO decalcifying solution.|| Serial sections were cut at 6μm and prepared alternately with hematoxylin-eosin and Mallory's trichrome stains.

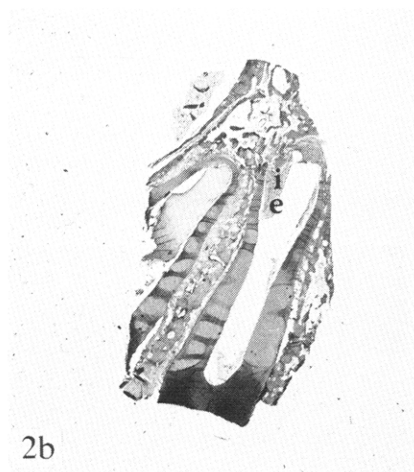
pical structures: c, cementum; v, lood vessel (Mallory's trichrome, orig nag × 100); 1e—higher magnification f dentin chips (d) encased within cementum (Mallory's trichrome, orig nag × 100); 1f—higher magnification of connective tissue (ct) (Mallory's richrome, orig mag × 200); 1g—igher magnification of cementum epair (cr) of root canal wall; notice eriodontal ligamentlike structure that as formed (Mallory's trichrome, orig nag × 200); 1h—epithelium (e) esembling cell rests of Malassez ocated within inflamed tissue, which s localized to area adjacent to gutta-percha seal (Mallory's trichrome, orig mag × 100).

Results

Histologic examination showed incomplete apexification of the control teeth containing calcium hydroxide-normal saline paste (Fig 1a and 1b). The control tooth filled with gutta-percha showed a periapical inflammatory response and epithelialization (Fig 2a, 2b, and 2c). A fourth control tooth, which had not been filled, showed some tissue ingrowth (Fig 3a and 3b); however, inflammation and resorption were evident.

Several teeth filled with collagen-calcium phosphate gel appeared to be revitalized with various forms of hard and soft connective tissue (Figs 1a, 1c, 1d, 1f, 3a, 3b, 3g, 4a, 4b, 4e, 4f, and 4g). In these cases, cementum, bone, and reparative dentin lined the wall of the root canal for most of its





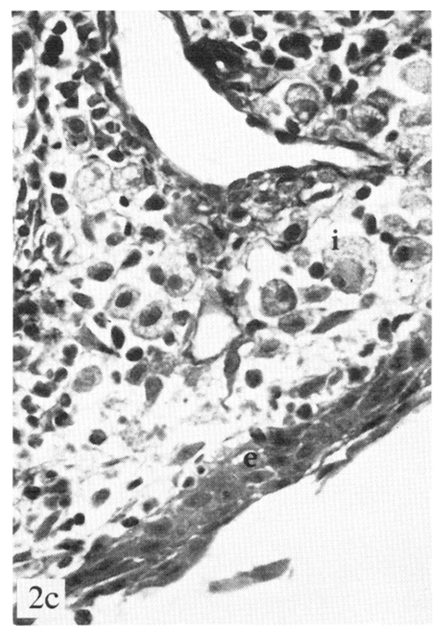
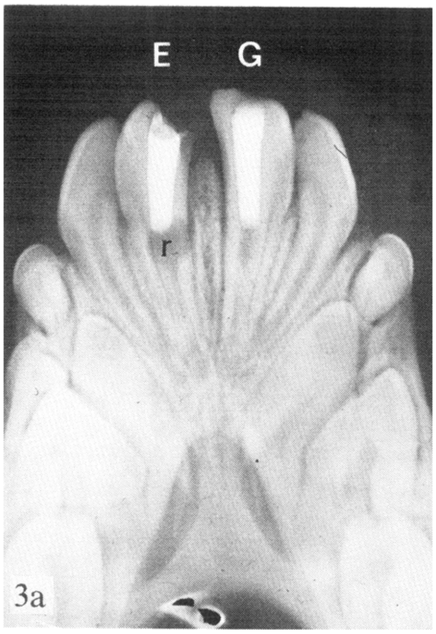
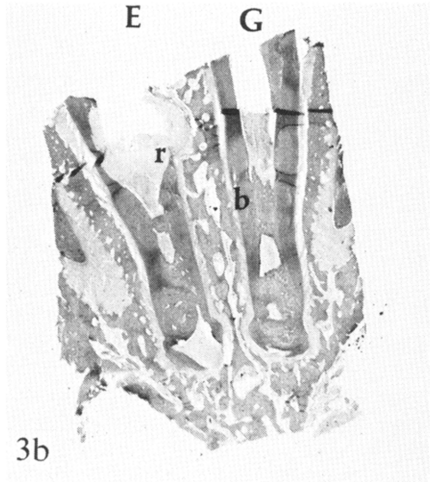
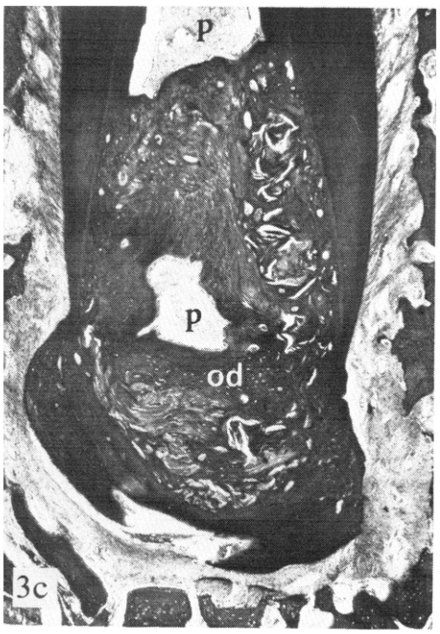
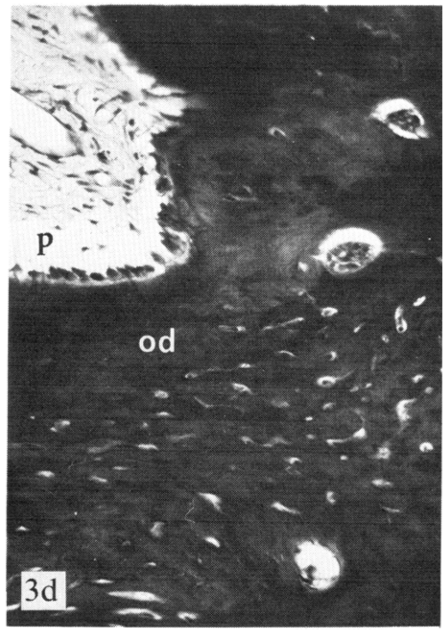


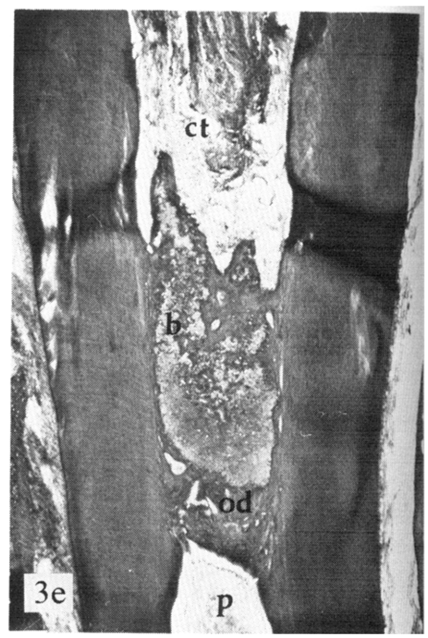
Fig 2a—Coronal portion of maxillary incisor filled with gutta-percha; sections cut obliquely (Mallory's trichrome, orig mag × 10); 2b—apical portion of same tooth in different serial section; inflamed periapical tissue (i) and epithelium (e) adjacent to gutta-percha are present (Mallory's trichrome, orig mag × 50); 2c—higher magnification of inflammatory cells (i) and epithelium (e) (Mallory's trichrome, orig mag × 200).

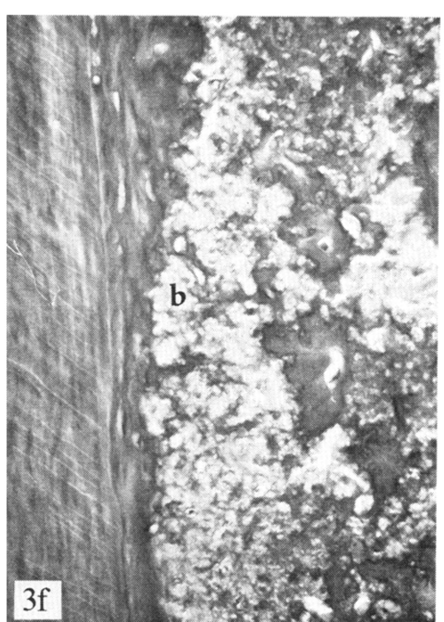












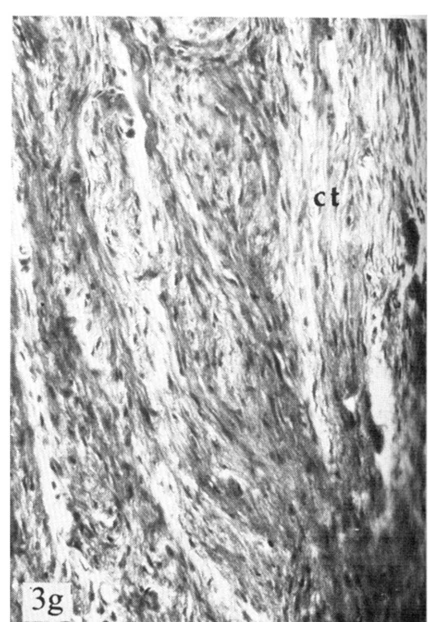


Fig 3a—E, radiograph of mandibular incisor whose canal was not filled; notice resorptive defect (r); G, gel-filled tooth showing radiopaque area within canal; 3b—corresponding histologic section: E, canal that was not filled; r, resorptive defect and inflammation; G, gel-treated tooth showing revitalization with various forms of connective tissue, including bone (b) (H&E, orig mag × 10); 3c—detail of G at apex: p, pulp tissue; od, osteodentin (H&E, orig mag × 50); 3d—higher magnification of Figure 3c (H&E, orig mag × 200); 3e—detail of G, tissue within root canal: p, pulp; od, osteodentin; b, bone; ct, connective tissue (H&E, orig mag × 50); 3f—higher magnification of bone (b) within canal (H&E, orig mag × 100); 3g—higher magnification of connective tissue (ct) within canal (H&E, orig mag × 100).

*Fig 4a—Pretreatment radiograph
open apex maxillary central inci
notice flaring apical morpholo
structure of apex; 4b—same tooth
weeks after pulp extirpation a
treatment with collagen-calcium ph
phate gel; notice constriction
apical architecture and apparent ap
ogenesis (a); 4c—histologic section
apex: f, original flaring root structu
c, cementum; a, apical extension
dentin; p, pulp (Mallory's trichro
orig mag × 50); 4d—higher mag
fication of apical root extension
and pulp (p) (Mallory's trichrom
orig mag × 200); 4e—serial secti*

ntaining coronal portion of same
oth; root canal is filled with various
rms of connective tissue: b, bone;
connective tissue; c, cementum
position on root canal wall (Mal-
ry's trichrome, orig mag × 10); 4f—
gher magnification of bone (b) and
eosteoid tissue (Po) within root canal
Mallory's trichrome, orig mag ×
0); 4g—higher magnification of
nnective tissue (ct) within root canal
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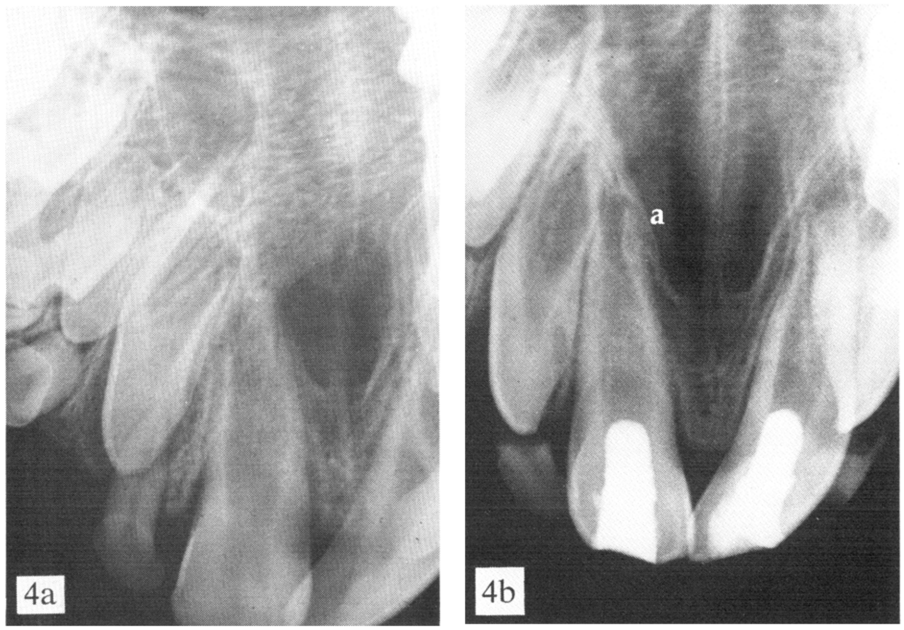

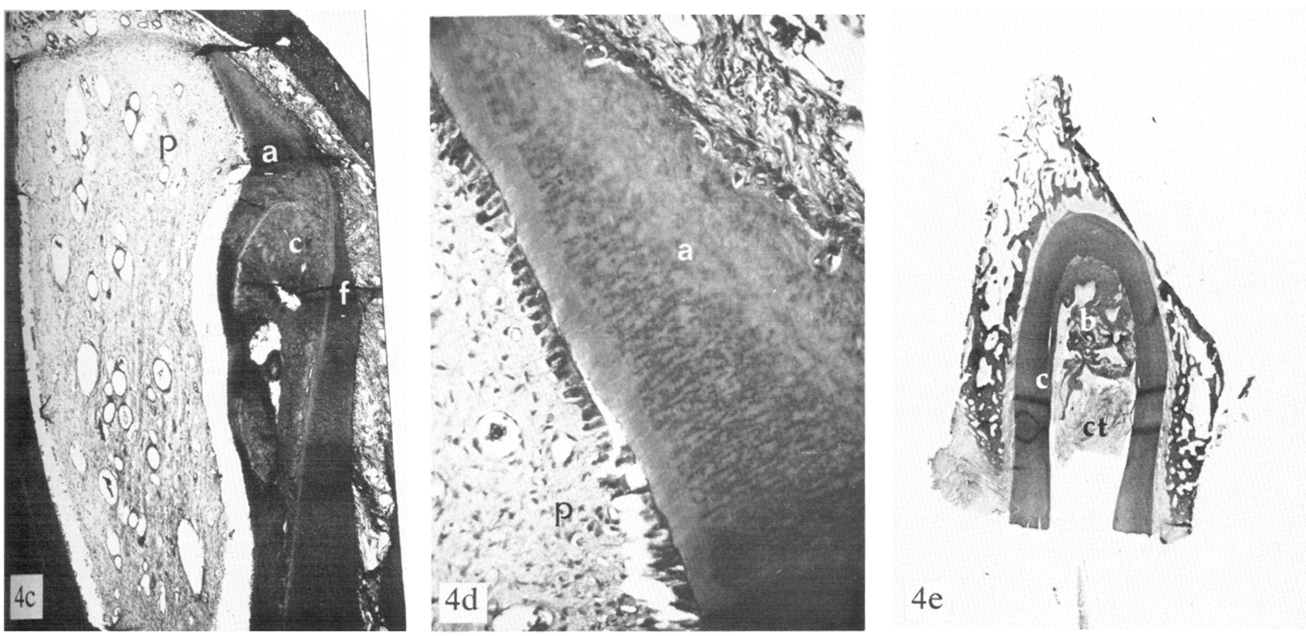

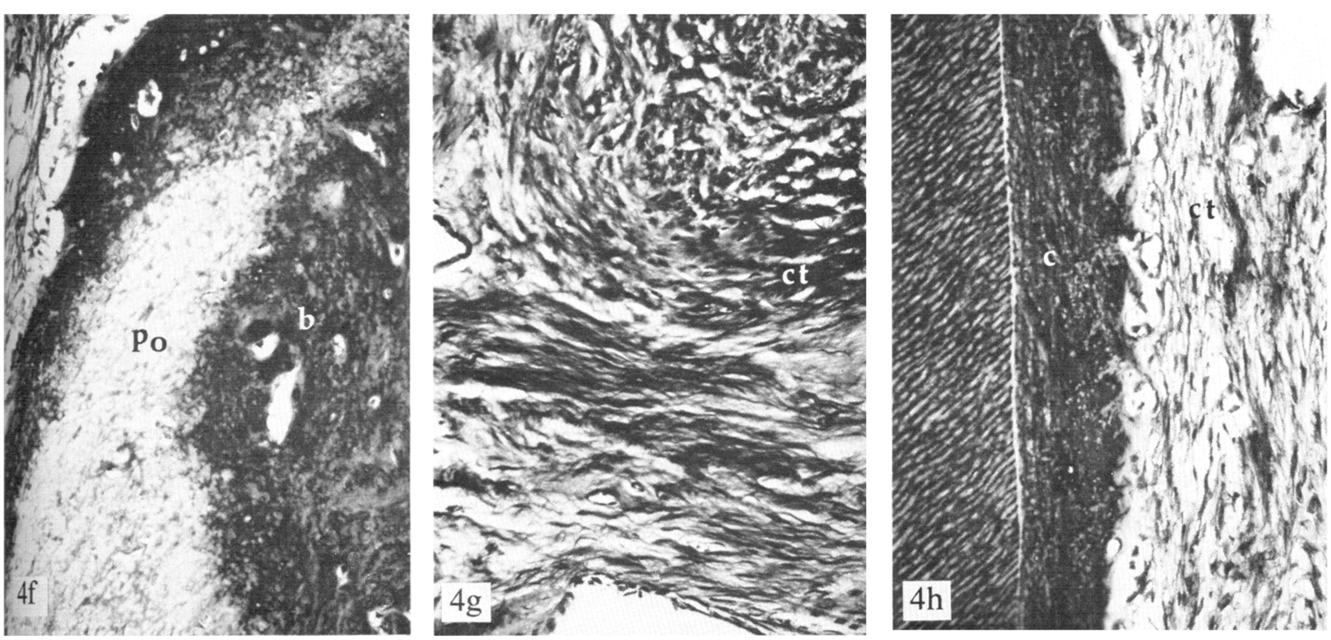

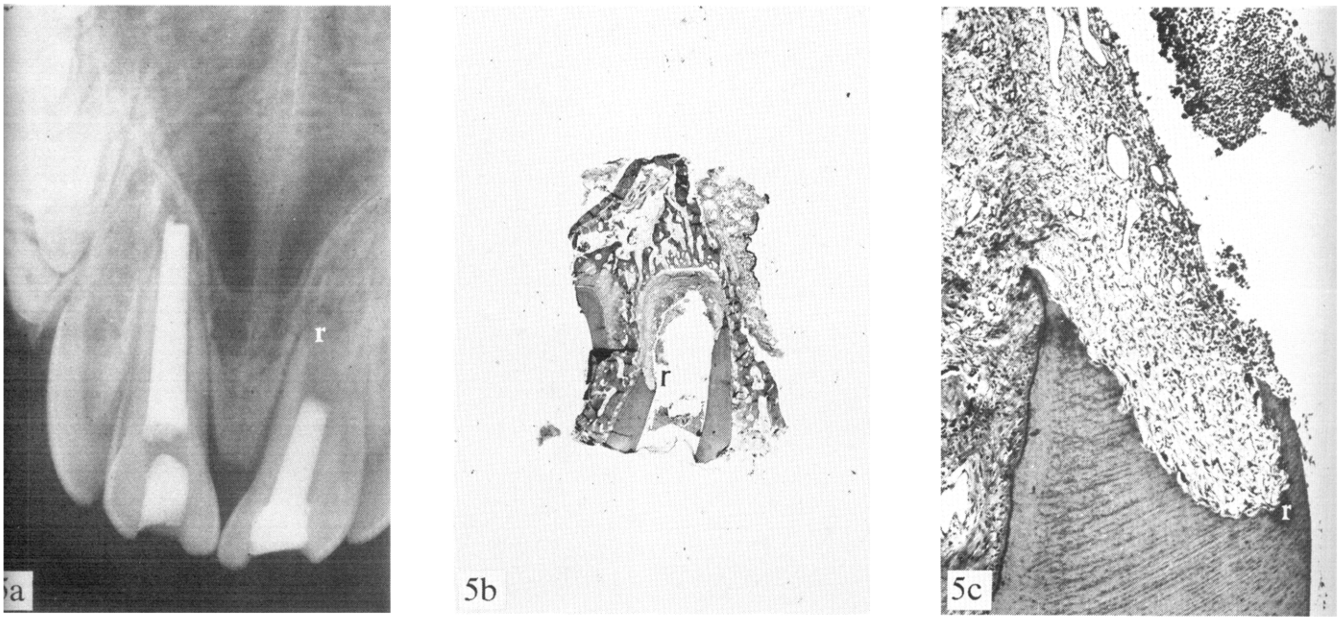

Fig 5a—Radiograph of gel-treated maxillary incisor 12 weeks after treatment; root resorption (r) is evident; adjacent tooth filled with gutta-percha is one whose histologic features are illustrated in Figure 2; 5b—histologic section of same tooth; root resorption (r) is evident (Mallory's trichrome, orig mag × 10); 5c—higher magnification of root resorption (r) (Mallory's trichrome, orig mag × 100).

length (Figs 1c, 1g, 3c, 3e, 3f, and 4h). Lacerated remnants of uninflamed pulp tissue produced substantial quantities of reparative dentin and osteodentin (Fig 3c and 3d). In one tooth, this resulted in a second apexogenesis and root lengthening (Fig 4c and 4d). Dentin chips routinely were found incorporated within new hard tissue formation (Fig 1e). Some gel-filled teeth in both groups were resorbed and contained inflamed tissue (Figs 1h, 5a, 5b, and 5c). Results of all antibody tests were negative.

Discussion

The results indicated that collagen-calcium phosphate gel has the capacity to induce revitalization of biomechanically debrided open apex teeth. Tropo-collagen molecules within the gel spontaneously polymerize at body temperature to form collagen fibrils that show 640-Angstrom cross-banding.[7] These fibrils are chemotactic for host fibroblasts and also form a microscaffold capable of supporting cellular migration (*Nature,* Aug 10, 1973, p 353). Dilute solutions of $CaCl_2$ and K_2HPO_4 combine within the gel to form hydroxyapatite crystals.[8] These crystals might serve as a nidus and seed connective tissue ingrowth, contributing to its ultimate mineralization.[9] Calcification of this tissue also appears to be influenced by local environmental factors such as inductive capacity of the root canal dentin.[10] Hard-tissue deposition onto the root canal surface might physiologically seal lateral and accessory canals, reducing the possibility of combined periodontal-endodontic lesions.

Remnants of lacerated pulp tissue appeared histologically normal, but elaborated large quantities of reparative dentin, altering their morphologic appearance. Root lengthening in one tooth suggests an apical shift in the position of apexogenesis, a result which appears contrary to current concepts of root morphogenesis.[11]

Absence of antigenicity to collagen within the gel corresponds to results obtained in other studies, indicating the weak antigenicity of this insoluble protein.[12]

Failures obtained through use of the gel might be attributed to bacterial contamination during debridement, leakage of the coronal seal, and blood clot incorporation into the gel. Attempts are being made to correct these factors in future experiments.

Summary

Collagen-calcium phosphate gel appears to be capable of inducing revitalization of pulpless open apex teeth in monkeys within 12 weeks. Connective tissue ingrowth and periapical tissue reorganization seem to be stimulated by the gel and influenced by local environmental factors. This material and this technique may prove to be a step in the direction of revitalizing human pulpless teeth.

*Sigma (no. C-3511), St. Louis.
†Sigma (no. T-1503), St. Louis.
‡L. D. Caulk Co., Milford, Del.
§Scientific Specialties, Ltd., Garden City, NY.
||Du Page Kinetic Lab, Inc., Downers Grove, Ill.

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