Comparative Medicine
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Vol 51, No 1
February 2001
Pages 70-74

Results of Crown-Height Reduction and Partial Coronal Pulpectomy in Rhesus Monkeys (*Macaca mulatta*)

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Background and Purpose: **In research facilities using non-human primates, crown-height reduction with partial coronal pulpectomy ("vital pulpotomy") is routinely performed on canine teeth of adult male monkeys to reduce self-trauma and the potential for injury to staff or cage-mates. Success of pulpotomy techniques in humans is reportedly 40 to 60%. Failure leads to chronic inflammation and pulp necrosis, which introduces variability in research animals, and may affect research results. The purpose of the study reported here was to determine failure rate of this procedure by evaluating clinical and radiographic findings at 3, 9, and 24 months after crown amputation and partial coronal pulpectomy of maxillary canines in adult male rhesus monkeys.**

Methods: **Forty-seven maxillary canine teeth from 24 adult male rhesus monkeys were treated by use of crown amputation and partial coronal pulpectomy, using standard dental technique. Follow-up clinical and radiographic examination was performed 3, 9, and 24 months after surgery.**

Results: **At three months after surgery, there was no clinical evidence of failure at any of the teeth. On the basis of radiographic findings, 2 of 47 teeth had failed and one was suspicious for early failure. At nine months, clinical evidence of failure was not apparent; radiographically, 5 of 44 teeth appeared to have failed and 3 others were suspect. Two years after surgery, failure of 41 teeth was clinically evident in two animals, with radiographic evidence of failure in five, and suspicion of early failure in an additional six.**

Conclusions: **The failure rate of crown amputation and partial coronal pulpectomy of canine teeth in adult male rhesus monkeys is high, and the chronic inflammation associated with this is cause for concern.**

In research facilities using non-human primates, crown-height reduction with partial coronal pulpectomy ("vital pulpotomy") is routinely performed on canine teeth of adult male monkeys to reduce self-trauma and the potential for injury to staff or cage-mates (1, 2). In human dentistry, vital pulp therapy of permanent teeth for treatment of recent tooth fractures with pulp exposure is controversial (3); success of pulpotomy techniques in humans is reportedly 40 to 60% (4). Failure leads to chronic inflammation and pulp necrosis. If teeth at which failure has occurred are not extracted or treated by total pulpectomy and obturation (root canal treatment), development of chronic inflammation in the periapical periodontal ligament and bone invariably occurs. Persistent inflammation in affected research animals introduces variability, and may affect research results. The purpose of the study reported here was to document clinical and radiographic findings at 3, 9, and 24 months after crown amputation and partial coronal pulpectomy of maxillary canine teeth in adult male rhesus monkeys.

When evaluating radiographs for evidence of endodontic disease, the pulp cavity and the periapical tissues are the primary focus. Vital pulp responds to trauma by formation of a tertiary dentin bridge that can be visualized radiographically. In addition, diameter of the pulp chambers and root canals of all corresponding teeth must be compared. A tooth with a uniformly relatively wider pulp cavity indicates that secondary dentin formation has ceased due to a lack of odontoblastic activity, which may be associated with pulpitis, fibrotic pulp, or more commonly, pulp necrosis. In immature permanent teeth with open apices, apexogenesis will cease if pulp necrosis occurs.

The inflammatory reaction in the periapical tissues following pulp necrosis is evidenced by changes in the integrity of the periapical lamina dura and surrounding periapical bone. The types of ensuing periapical pathologic changes may vary from acute or chronic periapical abscess, to periapical granuloma, condensing osteitis, osteomyelitis, and periapical cyst formation. Radiologic differentiation of these lesions is not always possible.

Periapical granuloma is the most common lesion found at the apex of a non-vital tooth (5). It may develop from acute or chronic periapical abscess, or may result directly from pulpal necrosis without abscess formation. Radiographic features of periapical granuloma include a well-circumscribed, round, radiolucent area with loss of the lamina dura (5).

Materials and Methods

Animals: All procedures were in conformance with the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Use and Care Committee. Twenty-four adult male rhesus macaques (*Macaca mulatta*), aged 6 to 13 years, were studied at the California Regional Primate Research Center (CRPRC).

The animals were housed in 0.5-acre outdoor cages (100 × 200 ft) holding 75 to 100 animals each. The animals were captured with nets, then brought indoors for the dental procedures. The animals were fed Purina Monkey Chow (25% protein, analyzed) with fruit and other supplements given according to CRPRC Environmental Enrichment Standard Operating Procedures. Drinking

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water was provided ad libitum by use of automatic devices. Water quality and purity were monitored. Animals were housed in compliance with established standards of the Federal Animal Welfare Act and the Institute for Laboratory Animal Resource.

Dental procedure: Food was withheld for 12 hours prior to the procedures, then animals were anesthetized by intramuscular administration of ketamine (10 mg/kg of body weight) or tiletamine-zolazepam (5 to 8 mg/kg). After endotracheal intubation, anesthesia was maintained with isoflurane and oxygen. The oropharynx was packed with exodontia sponges. The oral cavity was rinsed with povidone-iodine solution, then was sterilely draped. Sterile instruments and burrs, as well as aseptic technique, were used during the procedures. The procedures were performed by two veterinarians with dental experience, including a board-certified veterinary dentist and a resident in a veterinary dental training program.

Amputation of 5 to 7 mm of crown was performed on both maxillary canine teeth using a taper diamond bur on a high-speed dental handpiece, with continuous water irrigation. Four to 6 mm of coronal pulp was removed, using a pear-shaped diamond bur on a high-speed handpiece with continuous irrigation. Hemostasis was achieved by use of the blunt end of sterile paper points. A small amount of calcium hydroxide powder was placed on the exposed pulp, followed by application of hard-setting calcium hydroxide cement (Dycal: Dentsply International, Milford, Del.). The walls of the canals were cleaned, using a spoon excavator. The dentin and enamel were acid-etched for 30 sec. with a 37% phosphoric acid gel, then rinsed and dried. Following application of primer and light-cured adhesive bonding agent (3M Dental Products, St. Paul, Minn.), a light-cured hybrid composite restorative (Z100, 3M Dental Products) was placed in the access sites in 2 mm increments. The restoratives were finished with a 12-fluted carbide finishing bur on a high-speed handpiece. A final layer of light-cured adhesive was placed.

Postoperative care: After the procedure, intra-oral lateral view radiographs of the maxillary canines were obtained, using the bisecting angle technique (5), a dental x-ray machine, and size-4 dental x-ray film (Kodak Ultra-speed, Eastman Kodak Inc., Rochester, N.Y.). All subjects received intramuscular injections of cefazolin (20 mg/kg) and oxymorphone (0.1 mg/kg) after surgery. The animals were observed in hospital overnight before being returned to their outdoor cages. One maxillary canine tooth had a confirmed necrotic pulp prior to the procedure. This tooth was extracted and was not included in the study.

Three, 9, and 24 months after surgery, the subjects were immobilized by intramuscular administration of ketamine (10 mg/kg); in some instances, diazepam (0.5 mg/kg) was administered intravenously for additional sedation and muscular relaxation. The face and oral cavity were examined for presence of draining tracts or swelling. The teeth were examined for evidence of discoloration or restorative failure. Intra-oral, lateral view radiographs were obtained of both maxillary canine teeth as previously described. Radiographs were evaluated for apical closure (where appropriate); pulp cavity width, using a calibrated magnification loupe (Peak Scale Loupe 7×, Tohkai Sangyo Company, Ltd., Tokyo, Japan), evidence of periapical osteolysis, and evidence of dentinal bridge formation. Criteria used for assessment are summarized in Table 1.

Statistical analysis: Analysis of the data was performed, using Fisher's exact probability test (VassarStats, Richard Lowry 1998-2001, Vassar College, Poughkeepsie, N.Y.).

Table 1. Criteria for radiographic assessment

Assessment	Periapical Region	Pulp Cavity Width	Dentin Bridge
No evidence of failure	Intact lamina dura, no evidence of osteolysis; evidence of closure of immature tooth apex	decreased or unchanged	present or absent
Suspect early failure: two or more of the following:	Indistinct lamina dura, widening of periapical periodontal ligament; no evidence of closure of immature tooth apex	unchanged	absent
Failure	Periapical lucency; failure of immature tooth apex to close; apical resorption	decreased or unchanged	present or absent

Results

Forty-seven teeth from 24 individuals were treated by use of crown amputation and partial coronal pulpectomy. Three months after surgery, clinical examination revealed no evidence of failure (discoloration, draining tracts, or facial swelling) associated with any of the treated teeth. None of the 44 teeth evaluated at 9 months had clinical evidence of failure. Twenty-four months after surgery, two animals had clinical evidence of failure: a cutaneous draining tract was identified on the face of one monkey and a gray-discolored tooth was noted on the other. The radiographic findings are summarized in Table 2 and are illustrated in Figs.1 and 2. Immediate postoperative radiographs and 24-month postoperative radiographs of an immature permanent canine tooth are depicted in Fig. 1. Apical closure, deposition of secondary dentin along the pulp cavity walls, and formation of a dentinal bridge, together with absence of periapical osteolysis, are suggestive of continued pulp vitality. The radiograph in Fig. 2 demonstrates evidence of pulpal necrosis, with ill-defined periapical lucency and inflammatory resorption of the apex, identified 9 months after surgery. Lack of deposition of secondary dentin and absence of a dentinal bridge are also consistent with pulpal death. The tooth was clinically normal, and had appeared radiographically unremarkable at the 3-month follow-up.

Of the six teeth with confirmed failure at 24 months, one had been confirmed as failure at 9 months, three had been suspect at 9 months, and two had no evidence of failure at any of the previous examinations. The clinically discolored tooth had been radiographically assessed as a failure at 9-month re-examination, but there was no radiographic evidence of failure at the 24-month re-examination.

Of the 13 animals with immature permanent canine teeth (open or partially closed apices) at the time of the procedure, two were not available for long-term follow-up; nine animals (81.8%) had no evidence of failure at either treated tooth, and two had confirmed failures.

Of the 11 animals with closed canine apices at the time of the procedure, one was not available for long-term follow-up. Five animals (50.0%) had no evidence of failure at either treated tooth, two had unilateral or bilateral confirmed failures, and three had suspected failures at one or both treated teeth.

Thus, animals with immature canine teeth at the time of the procedure appeared to have lower long-term failure rate (18.2%) than did animals with mature canine teeth (50.0%). However, this difference was not found to be statistically significant (two-tailed probability = 0.183 calculated by use of Fisher's exact probability test).

Table 2. Radiographic assessment of treated teeth

	3-month radiographic recheck (47 teeth)	9-month radiographic recheck (44 teeth)	24-month radiographic recheck (41 teeth)
No evidence of failure	44	36	30
Suspect	1	5	5
Failed	2	3	6

Discussion

Management of captive primates in research facilities requires group-housing for social enrichment. After eruption of permanent canine teeth in Old World primate species, significant trauma can be inflicted on cage-mates and handlers. Historically, extraction of canine teeth was performed to minimize injury; however, extraction often led to complications such as bone loss, perforation into the maxillary sinuses, and jaw fracture (1, 2). Crown amputation with vital pulpotomy (partial coronal pulpectomy) was introduced as an alternative to extraction in the 1970s (1, 2).

This procedure aims to preserve pulp vitality and maintain function of the dentin-pulp complex. In human dentistry, vital pulp therapy is controversial, and use of vital pulp techniques is limited to primary teeth and immature permanent teeth (6) where conventional endodontic treatment (total pulpectomy) may be contraindicated. Calcium hydroxide, the material most commonly used for this procedure, allows continued root development and formation of a dentin bridge (7).

When evaluating vital pulp therapy radiographically, presence of a dentin bridge has been one of the criteria by which a procedure is judged successful (6, 8). However, formation of a reparative dentin bridge does not prevent pulp inflammation or necrosis (9), as the hard tissue is not a protective barrier due to the presence of tunnel defects and holes (10, 11).

Bacterial penetration is suspected to be the primary cause of inflammation and pulp necrosis following partial pulpectomy (8, 12, 13). Use of resin composite restorative systems rather than amalgam has addressed some concerns about bacterial micro-leakage. However, because calcium hydroxide does not adhere to dentin or to composite restorative materials and dissolves over time, softening of the adjacent composite interface and marginal bacterial leakage may occur (12). In the study reported here, longer-term follow-up revealed increasing failure rates; this is consistent with the theory that dissolution of the calcium hydroxide and marginal microleakage are the primary cause of failure. Future development of newer pulp capping and restorative materials may result in higher success rates for vital pulp therapy.

As an alternative to the use of calcium hydroxide, the "total etch technique" has been described for direct capping of exposed pulp with resin adhesives. When a multipurpose dentin bonding adhesive agent is applied to acid-etched dentin, an impermeable hybrid layer forms (6). Phosphoric acid, a dentin-etching agent, surprisingly does not appear to cause pulpal inflammation even when applied directly to pulp (13). Primer, adhesive, and resin composite systems also were found to be compatible with pulp tissue (14). However, use of this technique in primates resulted in pulp necrosis in 45% of experimental teeth, compared with 7% of controls (teeth that had been treated with calcium hydroxide prior to etching and placement of a restorative) (15). At that time, clinical studies of the total etch technique have produced conflicting results, with some authors reporting minimal inflammation (16), and others reporting severe responses (6, 15). In addition, adequate follow-up has not been performed to evaluate long-term effects of this technique on the pulp. Therefore, use of the total etch technique for direct pulp capping is not currently recommended (6).

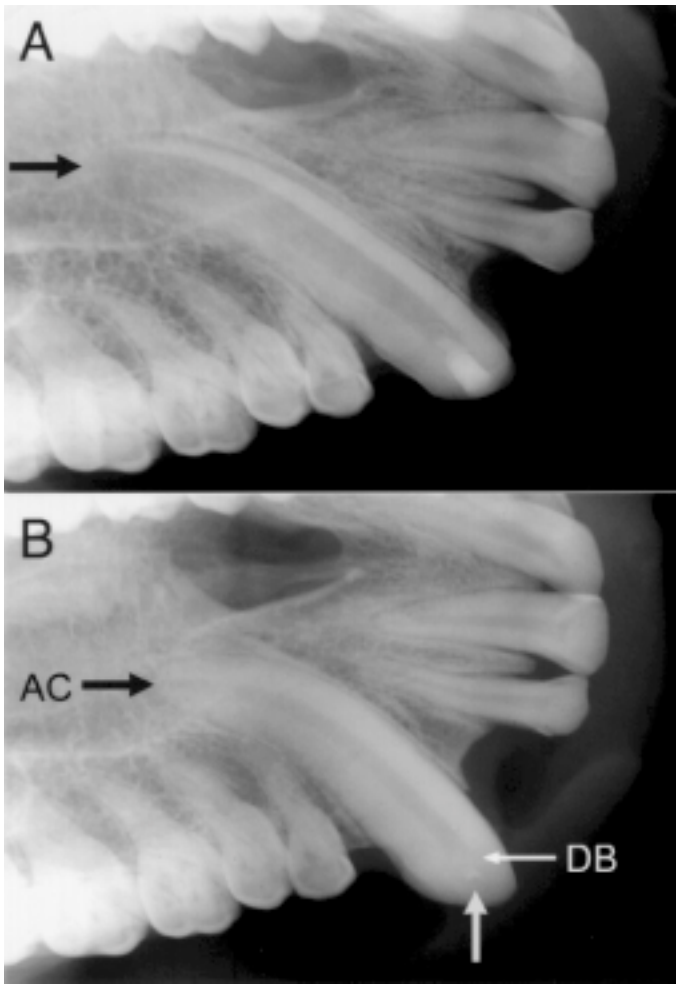

Figure 1. Right maxillary canine tooth. (A) Six and a half years old with open apex (horizontal arrow) at the time of the procedure; root canal width 2.8 mm. (B) Apical closure (horizontal arrow - AC) is evident at the 24-month follow-up examination; a faint dentinal bridge is present (horizontal arrow - DB); root canal width 1.4 mm. Notice how occlusal wear has reduced the width of the restoration (vertical arrow).

The ability of liners and adhesives to prevent bacterial ingress into pulp is critical in long-term success of vital pulp therapy (13). Despite adhering to dentin, glass-ionomer cements do not provide an adequate seal to prevent bacterial penetration into the pulp (13). Further research is required to identify a material that is biocompatible and has sustained antimicrobial activity and adhesive qualities. Materials such as mineral trioxide aggregate (MTA) have promise as pulp sealing agents (17). In addition, future development of restorative materials without polymerization shrinkage will eliminate marginal leakage and seal the pulp from bacteria (13).

In this study, 73% of teeth had no clinical or radiographic evidence of failure 24 months following crown amputation and partial coronal pulpectomy. Six teeth (15%) in four animals had periapical radiolucencies, indicating endodontic death. Of these teeth, all but one appeared clinically normal. One patient, with bilateral failures, had a draining tract on the left side of the face.

More subtle radiographic findings, suggesting early failure,

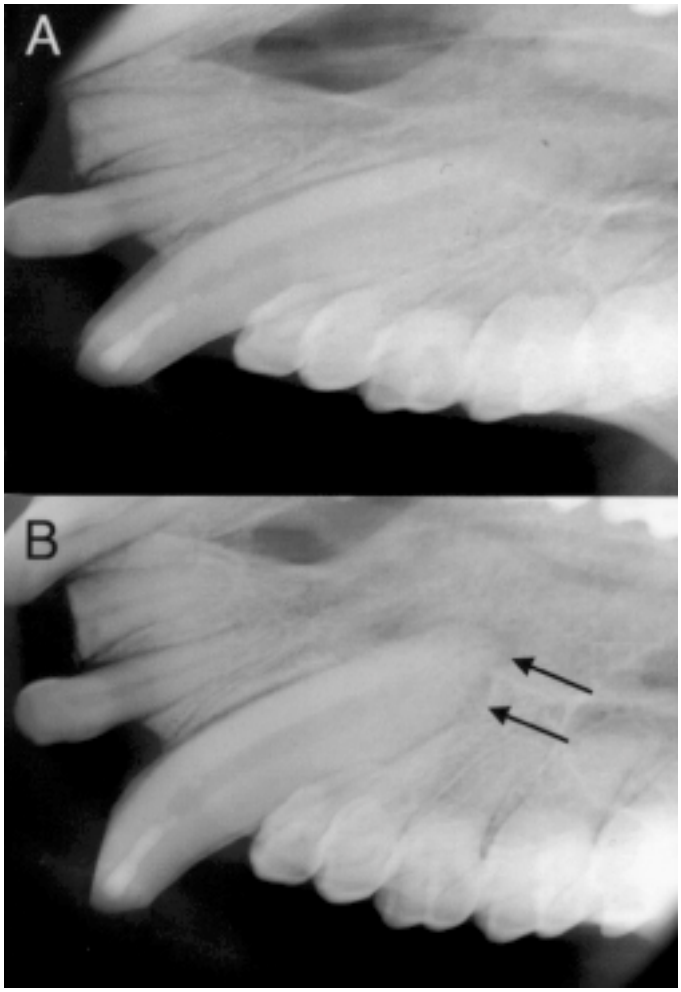

Figure 2. Left maxillary canine tooth. (A) Eight years old with closed apex at the time of the procedure; root canal width 1.5 mm. (B) Ill-defined and irregular widening of the periapical periodontal ligament space (arrow) is evident at the 9-month follow-up examination; no dentinal bridge is present; root canal width 1.5 mm.

were observed in an additional five teeth (12%), affecting an additional four animals. This indicates failure rate of 15 to 27%. However, correlation between clinical/radiographic findings and histopathologic findings has been reported to be approximately 80% (18); radiographic findings underestimate the number of actual failures as determined histologically.

In addition, obtaining radiographs of consistent quality, and interpretation of radiographic findings, is often difficult. Without proper use of the bisecting angle technique, the teeth will appear elongated or foreshortened on the film. Such images will not accurately reflect the width of the pulp cavity and may obscure changes in the periapical region. In addition, subtle changes in the radiographic appearance of the periapical tissues may go unnoticed by inexperienced personnel.

At 3, 9, and 24 months after surgery, the authors were unable to conclusively diagnose failure radiographically at several treated teeth. Three teeth that were questionable at 9 months were confirmed failures at 24 months, suggesting that even subtle changes in the periapical periodontal ligament indicate endodontic death. However, two teeth that were questionable at three and nine months, respectively, appeared within normal limits on subsequent follow-up radiographic views. Furthermore, three teeth interpreted as failures at three and nine months appeared radiographically normal at subsequent re-examinations. None of the changes seen on 3 month radiographic views were confirmed at 9- and 24-month examinations, suggesting that 3 months may be too early to accurately assess treated teeth. This, together with the appearance of newly diagnosed failures at 24-month radiographic evaluation, indicates the need for long-term clinical and radiographic follow-up of animals undergoing this procedure. Furthermore, in an investigation involving rhesus monkeys, 119 of 120 teeth "displayed normal periapical radiographic characteristics" despite histologic evidence of moderate to severe pulpal inflammation in 37 teeth and total pulpal necrosis in 9 teeth (9). Therefore, it is possible that those teeth that had radiographic evidence of failure represent only a fraction of teeth with chronic inflammation and/or necrosis.

In many laboratory situations, crown amputation and partial pulpectomy is performed by personnel with minimal dental knowledge and experience, without using aseptic technique. The animals are typically immobilized by administration of an injectable dissociative agent such as ketamine; inhalants are usually not used. In this study, procedures were performed by experienced veterinary dentists using sterile instruments and aseptic technique, with the animals under general anesthesia. Despite this, 8 of the 21 animals for whom 24-month follow-up radiographs were available had radiographic evidence of failure or suspected failure at one or more teeth. This suggests that, even under ideal conditions, 38% or more of adult male rhesus research subjects will have chronic inflammation following crown amputation and partial coronal pulpectomy. This introduces a possible source of significant variability in research results.

When indicated, crown amputation and partial coronal pulpectomy should be performed by veterinarians who have been properly trained in the technique. Pre- and postoperative intra-oral radiographs should be obtained. Sterile instruments and aseptic technique must be used. The animals must be adequately anesthetized, and immediate postoperative analgesia should be provided. Radiographic examination should be performed six months after the procedure and annually thereafter, with radiographs obtained and evaluated by experienced personnel. When failure is confirmed, the affected teeth must be treated by use of total pulpectomy or complete extraction.

Monitoring the status of these animals by use of dental radiography, and treating failures appropriately, will be extremely labor-intensive if a quarter or more of treated teeth are expected to fail. Alternatives to crown amputation include total pulpectomy (root canal treatment), extraction, and minimal crown-height reduction of canines without exposing pulp. As an alternative to vital pulpotomy, total pulpectomy has been described as "a quick, practical, one-step method for permanently disarming nonhuman primates" (19). The authors describe use of broaches to remove pulpal tissue, followed by rinsing the canal with saline or water, and drying by use of paper points. The root canal filling material was a paste composed of zinc oxide and eugenol, paraformaldehyde, bismuth compounds, titanium oxide, and barium sulfate. Twelve-month radiographic re-examination revealed "no signs of infection." However, long-term follow-up was not reported. It should be noted that proper cleaning, shaping, and obturation of the root canal is a multi-step procedure. Total pulpectomy requires obtaining radiographs prior to, during, and after the pro-

cedure to ensure adequate cleaning and obturation. The canal walls must be filed to ensure removal of all organic material, and the canal is typically rinsed with sodium hypochlorite for bactericidal action. After drying, the canal is obturated with a permanent, biologically inert material (typically gutta percha) (20). The paste used in the aforementioned procedure (19) was neither permanent nor biologically inert. Zinc oxide-eugenol cements lose volume over time due to dissolution in tissues (21). Paraformaldehyde may cause irritation in the periapical tissues and may be systemically absorbed (20). Root canal treatment is not "quick" and easy, but a time-consuming and sometimes complicated task if performed correctly.

As mentioned previously, extraction is time consuming and is associated with postoperative complications. Slight reduction of crown-height, using a low-speed dental handpiece, without exposing pulp, will induce formation of tertiary dentin within the pulp. This procedure is not time consuming, and could be performed during routine physical examination. If 1 to 2 mm of crown is removed every six months for two years, the tooth may eventually be reduced in height by 4 to 8 mm without exposing pulp tissue.

However, the need to perform any of these procedures should be re-evaluated, because to the authors' knowledge, studies documenting the benefits of crown-height reduction have not been published. Thus far, there has been no scientific evidence that the severity or frequency of bite injuries is reduced following crown amputation and partial pulpectomy of canine teeth. Until such evidence is documented, this procedure should not be performed routinely for colony management, but should be reserved for animals who have displayed repeated, severe aggression toward handlers or cage-mates.

Acknowledgment

We thank Andrew Hendrickx and Jeffrey Roberts of the California Regional Primate Research Center for permission to conduct this investigation, which was part of the Center's pilot project and venture program (base grant No. RR00169). We also express our appreciation to Cheryl Terpak, RDH, to Sarah Davis, and to the staff of Therapeutics, Romy Daquioag in particular, for their assistance in executing this project.

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