

The Vervet Monkey (*Cercopithecus aethiops*) as an Experimental Model for Pulpal Studies

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OPSOMMING

Die fisiese en chemiese eienskappe van 'n potensiële herstel materiaal kan nie langer van die biologiese oorweging van die mondomgewing geskei word nie. Hierdie eienskappe van 'n moontlike herstel materiaal behoort op drie progressiewe vlakke getoets te word, waarvan één die voorlopige histologiese ondersoek in geskikte diereweefsels insluit. Die hond, rot, vark en aap (*Macaca irus*, *M. mulatta* en *M. nemistrina*) is almal reeds herhaaldelik vir hierdie doel gebruik. Die blouaap (*Cercopithecus aethiops*) is vrylik beskikbaar in Suid-Afrika vir navorsingsdoeleindes en die tandmorfologie en histologie is soortgelyk aan dié van die mens. Die werksprosedure is beskryf en kaweite in kontra-laterale kwadrante is herstel met 'n kontrole-materiaal en 'n herstel materiaal respektiewelik. 'n Eenvoudige deursyfering tegniek is gebruik om voldoende fiksasie van die pulpa-weefsels te verseker. Die diere is op drie verskillende tye om die lewe gebring om sodoende die volle omvang van die pulpa-reaksie te kan dokumenteer. Die tande is sjirurgies verwyder; die tegniese prosedure en histologiese eienskappe is beskryf. Daar is tot die gevolgtrekking geraak dat die blouaap uiters geskik is vir eksperimentele doeleindes in die voorlopige evaluasie van pulpa-reaksies van herstel materiale.

SUMMARY

The physical and chemical properties of a potential restorative material can no longer be separated from the biological considerations of the oral environment. The biological properties of a candidate material should be tested at three progressive levels one of which includes preliminary histological evaluation in appropriate animal tissues. The dog, rat, pig and monkey (*Macaca irus*, *M. mulatta* and *M. nemistrina*) have been used extensively for this purpose. The vervet monkey (*Cercopithecus aethiops*) is readily available for experimental studies in South Africa and the dental morphology and histology are similar to those of man. The operative procedures are described and cavities in contralateral quadrants restored with a control material and a restorative material respectively. A simple perfusion technique was employed to ensure adequate fixation of the pulpal tissues. The animals were sacrificed at three specific time intervals so that the full range of pulpal responses could be documented. The teeth were removed surgically, the processing technique described and the histological features presented. It is concluded that the vervet monkey is an excellent experimental model for the initial evaluation of pulpal responses elicited by restorative materials.

INTRODUCTION

Until recently little consideration has been given to the interaction between dental restorative materials and their host tissues, dentine and pulp. This is surprising because, according to Baume (1961), Gysi described the effect of zinc oxide on the pulps of human teeth as early as 1900. The early investigations were not well controlled but since 1950 improved techniques and the standardization of testing procedures have resulted in a more scientific approach towards the study of pulpal responses elicited by restorative materials. This is evident from the work of pioneers in this field namely Shroff (1952), Kramer (1954), Langeland (1957), Brännström (1968) and Stanley (1968 a, b).

Phillips (1965) pointed out that the physical and chemical properties of a potential restorative material can no longer be separated from the biological considerations of the oral environment. As far as the biological properties of a candidate material are concerned, the Bio-materials Research Advisory Committee of the National Institute of Dental Research (1966) recommended that a material should be tested at three progressive levels:

- (a) Toxicity tests such as the LD₅₀ test and mucosal irritation tests.
- (b) Preliminary histological evaluation in appropriate animal tissues.
- (c) Biological pulp studies in human teeth after the animal studies have proved a candidate material acceptable.

The final study on human material is essential because both the clinical and biological acceptability of a product can be assessed (Baume, Fiore-Donno and Holz, 1971). To facilitate the statistical evaluation of results a uniform series of experiments should be performed with only one variable (Brännström and Nyborg, 1969). The experiments involve comparison between a test and a control material and ideally intra-individual comparisons between contralateral pairs of teeth should be made. The cavities in the contralateral pairs of teeth should be prepared and restored at the same appointment and subsequently extracted at the same time after a specific time interval (Stanley, 1968 b). Stanley and Swerdlow (1964) suggest that at least 75 to 100 teeth extracted at three time intervals should be used in such a trial. By studying the histological sections at three time intervals; after a few days, after 10-14 days and after four to six weeks, the initial response, the further development of the lesion and the resolution can be observed. The full range of pulpal response can be documented and the investigators provided with a dynamic sequence of events.

Because of the problems associated with such a large

scale experimental investigation in human material, the study of pulpal responses by means of preliminary animal phase investigations is more practical. The value of pulpal studies in experimental animals has often been questioned because of the variation in animal species and the operative procedures used. Lee and Ocumpaugh (1970), in a review of species response to conventional restorative materials, concluded that the pulp of the rat, dog, monkey and human appear to respond in a similar way to a specific restorative material.

The primates most commonly used in pulpal and biological studies are the rhesus macaque (*Macaca mulatta*) (Mitchell, Buonocore and Shazer, 1962; Adams and Lord, 1971; Safer, Avery and Cox, 1972), the pig-tailed macaque (*Macaca nemistrina*) (Ocumpaugh, 1970) and the cymologous macaque (*Macaca irus*) (Cohen, 1971). The vervet monkey, (*Cercopithecus aethiops*), is readily available for experimental studies in South Africa. The anatomy and histology of its dentition have been fully described by Ockerse (1959, 1963). The similarity of the dental morphology and histology of this primate and man has prompted this investigation.

MATERIALS AND METHODS

Adult monkeys in which the third molars had erupted, were used in this study. The operative procedures were carried out under general anaesthesia induced with ether and maintained with Sodium Cyclonal* administered intravenously at a dosage of 22 mg/Kg of bodyweight. Smaller, additional doses were administered intramuscularly if required.

Buccal class V cavities were prepared in 20 teeth in each animal. The incisors, premolars and first molar teeth in each quadrant were used. The canine teeth were omitted because they cannot be easily extracted. The cavities were cut with a No. 2 inverted cone tungsten carbide bur (I.S.O. size 010) run at a low speed with adequate water cooling (Fig. 1). A Kavo Supra combi Contra Angle Shank 98 handpiece** connected to a W + H mobile dental engine*** capable of a maximum speed of 12,000 rpm was used. The cavity preparations were done under light pressure at a speed of approximately 5,000 rpm. Cavities prepared in the upper jaw of one quadrant were filled with a test restorative material and those in the contralateral quadrant with Nobetec. Nobetec**** is a modified zinc oxide-eugenol cement and was chosen as the control material. The filling procedure was reversed in the lower jaw of each animal.

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** Kaltenbach and Voigt, 795 Biberach/Riss, West Germany.

*** Dentalwerk; Salzburg; Austria.

**** Bofors, Nobel-Pharma, Sverige.

The animals were sacrificed at three specific time intervals after the operative procedures, namely four days, two weeks and six weeks. The primates were sacrificed after their kidneys had been removed and the renal vessels clamped. The kidneys are used for the production of anti-poliomyelitis-vaccine and thus obviates the sacrifice of animals purely for pulpal studies. The thoracic cavity was opened by incising the abdominal muscles and diaphragm along the margin of the costal arch. The thoracic aorta was dissected free from its vertebral attachment and was sectioned just cranial to the diaphragm. The cranial section of the aorta was cannulated and the cannula tied in place. The superior vena cava was sectioned and one litre of warm (37° C) physiological saline was passed into the cannulated aorta and perfused all the tissues receiving an arterial blood supply from the thoracic aorta. After about 750 ml of saline had been passed into the aorta, a clear venous perfusate drained from the sectioned superior vena cava.

The jaws of the monkeys were opened maximally and maintained by inserting a plastic tube longitudinally between the canine teeth to facilitate extraction of the teeth after fixation by perfusion. The cannulated vascular system was then perfused with a buffered 10% formol saline solution. After the latter perfusion the buccal and lingual alveolar plates were removed from the upper and lower jaws with an osteotome and the teeth gently elevated from their sockets (Fig. 2).

The extracted teeth were each allocated individual random numbers obtained from a table of random numbers (Scientific Tables, 1962) and suspended in buffered 10% formol saline for three days. The teeth were decalcified in either buffered 5% formic acid solution at room temperature or in 0.5M E.D.T.A. buffered to a pH of 7.4 at 60°C (Nikiforuk and Sreebny, 1953). Decalcification in the formic acid solution was carried out by suspending the teeth in individual containers held in a shaking water bath. Decalcification in E.D.T.A. was carried out in an oven at 60°C with daily shaking for a few minutes.

The specimens were transferred into fresh decalcifying solutions at intervals of 3 days for two weeks and then, to facilitate the final decalcification, the mesial and distal surfaces were carefully trimmed away taking care not to expose the pulps of the teeth. The teeth were further decalcified until X-ray examination revealed that complete decalcification had taken place. This usually required an additional week with both techniques. The restorative materials still present in the teeth were carefully dislodged with a needle. The teeth were processed in the usual way and finally embedded in wax under vacuum. Transverse bucco-lingual serial sections were cut at 7 μ m and stained

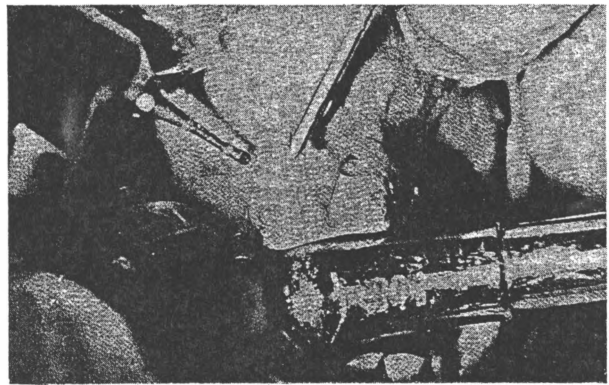


Fig. 1. The preparation of buccal class V cavities.

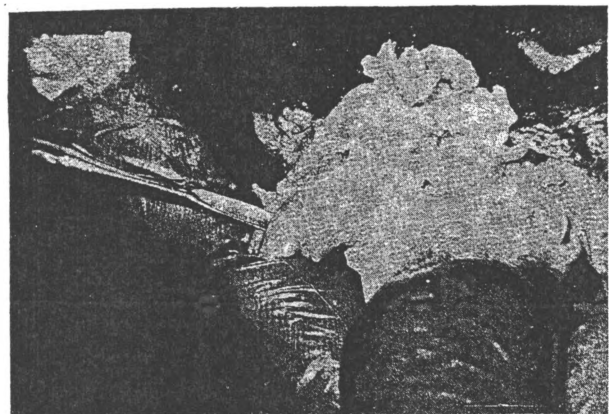


Fig. 2. Elevation of exposed teeth from their sockets after removal of the buccal and lingual alveolar plates.

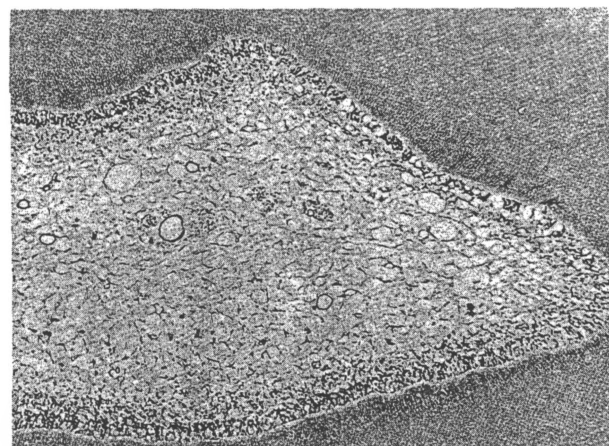


Fig. 3. Histological appearance of central incisor pulp decalcified with buffered 5% formic acid. H and E x 100.

with either haematoxylin and eosin or Masson's Trichrome stain.

RESULTS

The method used for preparing the teeth for histological examination provides adequate preservation of the delicate pulpal structures. There is no withdrawal of the odontoblasts from the dentine and the cellular details of the odontoblasts, fibroblasts and fine fibrillar network and bloodvessels are clear (Fig. 3).

The sections prepared from specimens decalcified with either of the two techniques are comparable with the exception that the cellular details are more clearly defined in the specimens decalcified with E.D.T.A. at 60°C (Fig. 4). The predentine is visible and the cell-free zone of Weil is well defined. Vacuoles are present in a small proportion of the odontoblasts and this is a constant finding in all the sections examined.

A higher magnification demonstrates the spindle-shaped or columnar odontoblasts with most of the nuclei situated at the ends of the cells furthest away from the dentine. The cytoplasmic processes of the odontoblasts extend into the dentinal tubules (Fig. 5).

In this study, the "effective depth", a term suggested by Shroff (1952) to describe the depth of cavities and recorded as distance from pulp to floor of cavity, can be controlled within reasonable limits. The effective depth in a tooth restored with the control material is 0.31 mm (Fig. 6). In this section the non-irritating effect of the control material, a modified zinc oxide-eugenol cement, is evident. The effect of this material on the pulp of the vervet monkey is similar to that reported for zinc oxide-eugenol on the pulps of other experimental animals and man (Lee and Ocumpaugh, 1970). The pulpal response of the restorative material being evaluated in this study has not previously been investigated in experimental animals and man. These results are to be published in a subsequent paper.

DISCUSSION

The main advantage of using experimental animals for assessing the pulpal response to restorative materials is that experimental variables can be reduced to a minimum. The preparation and restoration of cavities in five teeth in each of the four quadrants in the vervet monkey can be accomplished in one session and all the restored teeth can be extracted at the same time after a specific time interval. By placing the test and control restorative materials in the teeth of contralateral segments respectively, the pulpal response can be evaluated under comparative environmental conditions. Six animals, two of which were sacrificed at a specific time interval, provide adequate data for statistical evaluation of the results. One factor, however, could not be controlled in this study and that was the age of the experimental animals. Adult monkeys with their third molars erupted were used in this investigation.

The most important single factor in determining the pulpal response to a restorative material is the remaining dentine thickness between the floor of the cavity and the pulp chamber (Stanley and Swerdlow, 1964). This thickness can be kept fairly constant by

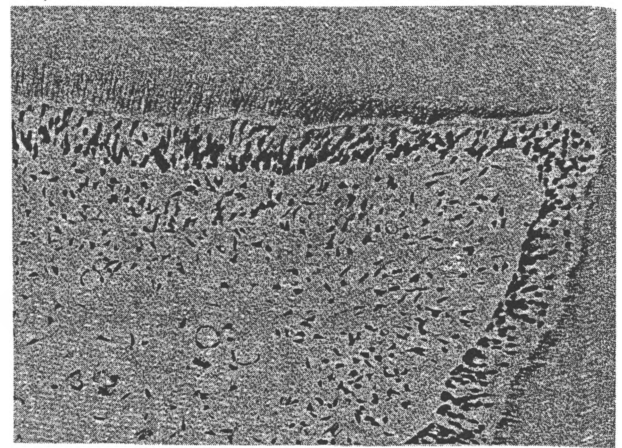


Fig. 4. Clearly defined cellular details in specimen decalcified with 0.5M E.D.T.A. at 60°C. Note vacuoles in odontoblasts. H and E x 250.

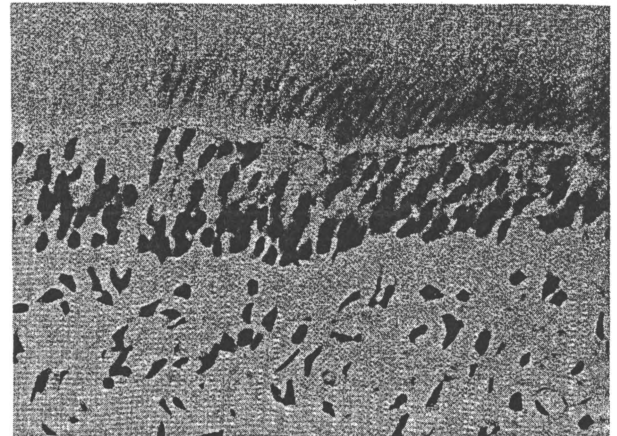


Fig. 5. Spindle-shaped and columnar odontoblasts with cytoplasmic processes extending into dentinal tubules. H and E x 640.

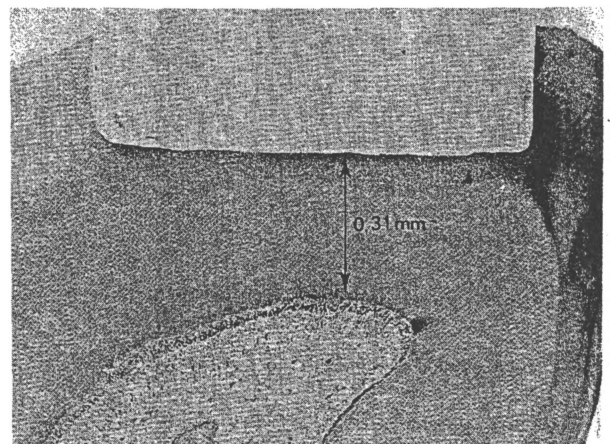


Fig. 6. The effective depth in an anterior tooth restored with Nobetec. Note the non-irritating effect on the pulp. H and E x 50.

careful preparation of the cavities. Stanley (1968 a) states that all specimens with a remaining dentine thickness greater than 2.0 mm should be eliminated in a study of this nature. Since the average buccolingual width of the necks of the incisors and premolars of the adult vervet monkey is 4.0 mm and

5.0 mm in the first molar (Ockerse, 1959) all the cavities prepared in this species will meet this requirement. Pulp exposure is rare and in only three of the 120 teeth restored in this study were the pulps exposed.

The non-irritating properties of zinc oxide-eugenol are well-documented and recommended as a control material (Shroff, 1952; Stanley, 1968 a; Lee and Ocumpaugh, 1970). These properties are attributed to the neutral pH of the mixture and the initial adaptation of this material to the cavity margins which is superior to that of any other cementing or restorative material (Phillips, 1965). The authors used Nobetec in this investigation. The material is a modified zinc oxide-eugenol cement containing certain resins and sythetic fibres. The adhesion of Nobetec was found to be superior to that of conventional zinc oxide-eugenol cements and in only one of the 60 control teeth restored did the cement become dislodged prior to sacrifice.

A major problem in the study of pulpal responses may be the slow or inadequate penetration of the pulpal tissues by the fixing solution. Marsland and Shovelton (1970) and Adams and Lord (1971) recommend the clipping of the roots of the teeth immediately after extraction and prior to fixation to increase the pulpal exposure to the fixative. Sorenson and Gatewood (1966) studied the penetration of 10% neutral formalin solution labelled with ^{14}C formaldehyde in freshly extracted human teeth. They found that intact teeth or teeth in which 5 mm of the root apices were clipped, required several hours of fixation before complete formalin penetration of the pulp chambers occurred. These authors did however find that rapid and extensive penetration of the pulp chambers occurred in teeth in which the entire mesial or distal surfaces were shaved with adequate water cooling. To exclude the possibility of autolytic breakdown of pulp tissues due to inadequate penetration of the fixative or unpredictable pulpal changes which may arise from a shaving process, a simple perfusion technique was used in this study.

According to Shroff (1952), profound differences in histological appearances may be brought about by variation in extraction techniques. He states that difficult extractions may result in an artificially created hyperaemia and suggests that surgical dissection of the teeth should be the method of preference with experimental animals. For this reason the canines of the vervet monkey were not included in this study and all the experimental and control teeth were removed surgically.

There are many conflicting opinions in the dental literature as to the effects of cutting procedures on the dentine and pulp (Stanley and Swerdlow, 1959; Langeland and Langeland, 1968, 1970). The

consensus of opinion at present seems that minimal trauma is produced if adequate cooling is maintained during the cutting procedure. In this study the cavities were prepared using light pressure and a drill speed of approximately 5000 rpm with ample water cooling.

The significance of vacuoles in the odontoblasts also remains a controversial matter. Shroff (1952) states that although the exact significance of vacuolization changes in the odontoblasts of experimental teeth has not been determined, there is no reason to dismiss them as of no consequence because the vacuoles suggest accumulation of intercellular oedema fluid. Lee and Ocumpaugh (1970), however, reported that occasional vacuoles have been noted in odontoblasts of untreated human and monkey teeth. A similar observation was made in the present study (Fig. 4). The occurrence of occasional vacuoles in the odontoblasts of untreated vervet monkey teeth could be a normal histological feature or artefacts produced during the preparation of the material for histological examination.

The possibility of a biased assessment of pulpal responses arising from a knowledge of the origin of the histological specimens was ruled out by coding the specimens with random numbers (Kramer, 1960; Brännström and Nyborg, 1969). To comply with these recommendations, individual specimens were allocated a random number immediately after extraction. These numbers accompanied each specimen throughout processing and histological evaluation.

CONCLUSION

The vervet monkey provides us with an excellent experimental model for the initial evaluation of pulpal responses elicited by restorative materials provided that adequate precaution is taken to eliminate the variable factors which may also influence pulp responses.

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