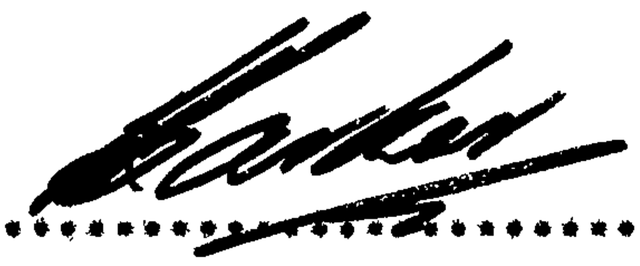
EXPERIMENTAL ENDODONTICS

Research into experimental endodontics using the dog as a subject.

A thesis submitted to the University of Sydney in support of my candidature for the degree of Doctor of Philosophy.



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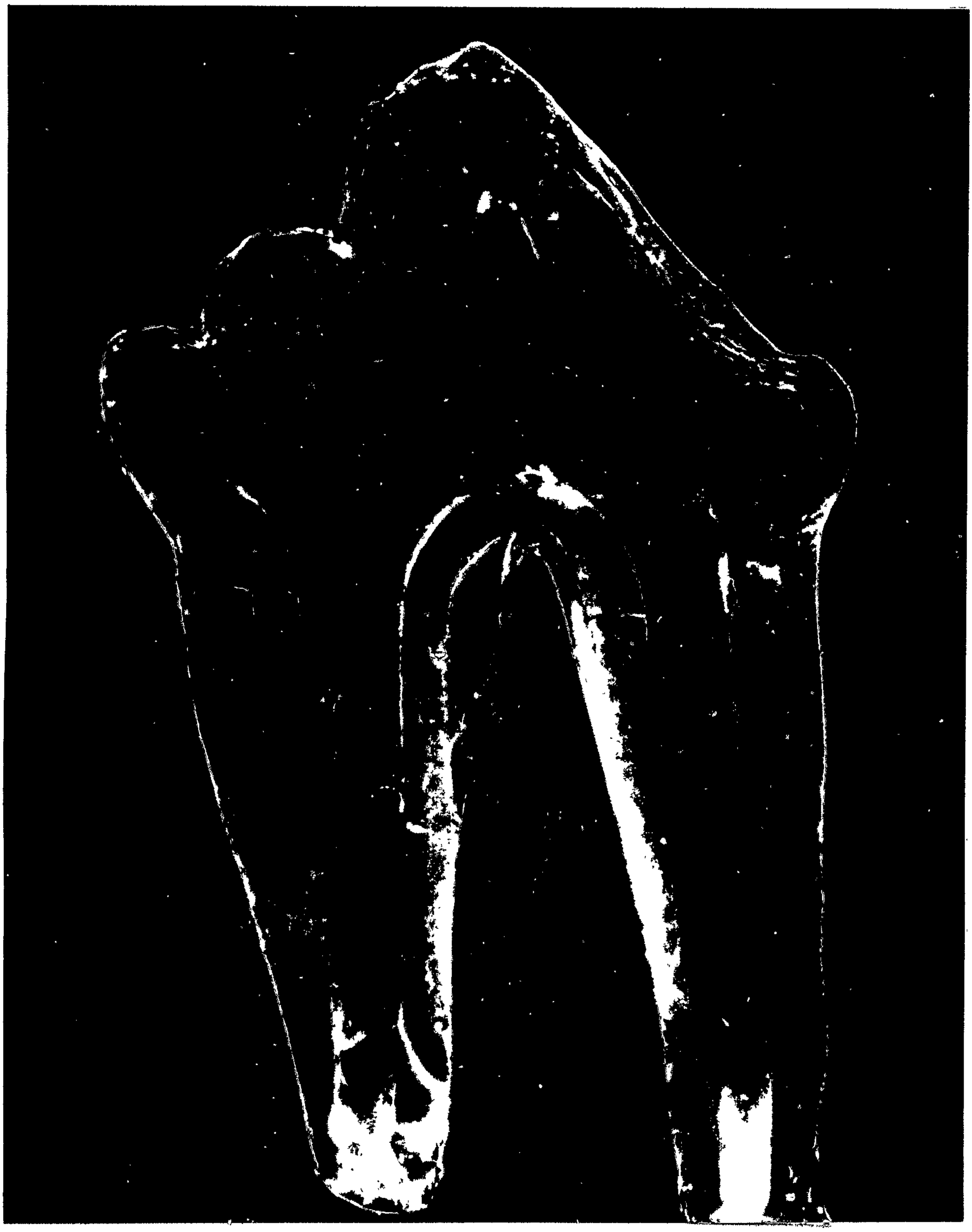
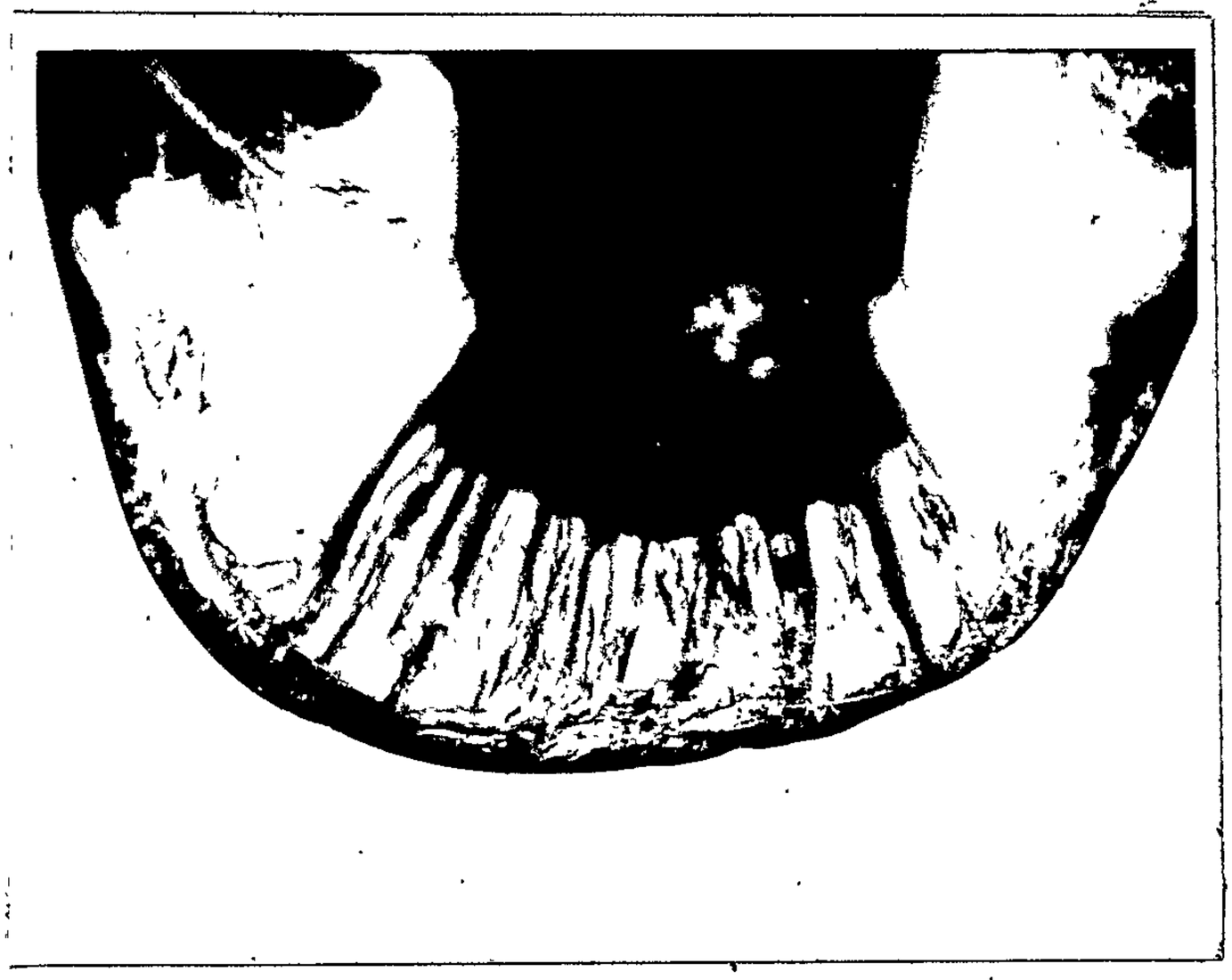
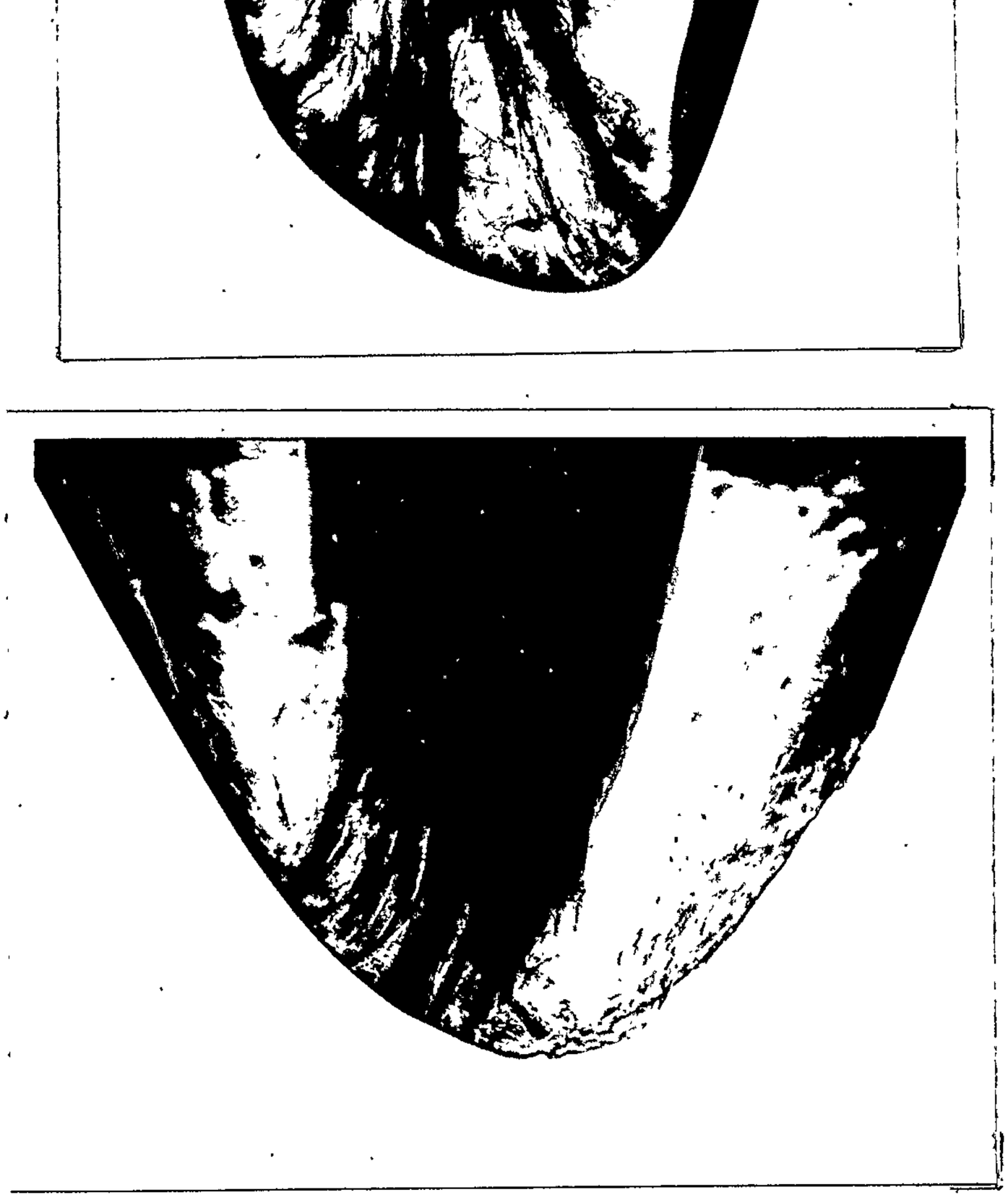


The mandibular fourth right premolar of the dog. Translucent replica demonstrating pulp anatomy.(10)





The root apex in teeth of the dog. Translucent replicas demonstrating the apical ramifications of pulp tissue.(10)

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EXPERIMENTAL ENDODONTICS

Introduction

Endodontic treatment is undertaken to promote the healing of injured pulp or periapical tissue, and diverse drugs and materials are available for application to the exposed pulp and for use in root canals. Unfortunately the relative effectiveness of these various materials in counteracting pathological change and in creating an environment conducive to tissue repair has by no means been definitively assessed. The evaluation of therapeutic measures employed in the practice of endodontics is therefore a pressing need.

Successful therapy is usually indicated by an absence of symptoms together with the preservation or restoration of an intact lamina dura, and thus long term clinical and radiographic observations would clearly have their value. Nevertheless, precise information concerning the reaction of pulp or periapical tissue to a particular procedure is best derived from the microscopic evidence of histologic examination.

The histologic study of human teeth which have been successfully treated endodontically is made difficult by the fact that such teeth cannot normally be recovered from adult patients unless a prosthetic requirement dictates their extraction at a later date. Teeth extracted for orthodontic reasons provide some material for this research but the observation period is necessarily short and young teeth do not necessarily reflect the changes which occur in older teeth. It is also apparent that, while some information about the response of human pulp to capping or pulpotomy is obtainable with great difficulty, it is virtually impossible microscopically to examine the responses of human tissue beyond the apex of treated teeth unless those teeth are fortuitous findings in cadaver material.

Nygaard Ostby[184] was so persuaded that the histologic examination of the apical part of the root and of the apical periodontium was the only method of obtaining knowledge about reactions to surgical operations in the root canal, that he described a technique for the removal of blocks of tissue from the patient's jaw. The understandable difficulty in obtaining consent for such surgery however, makes it more practicable to supplement the limited information available from human sources with that from animal experimentation, even though Ostby himself regarded the results from animals as questionable.

It is one of the objects of this thesis to illustrate the extensive range of endodontic investigations which may be undertaken in a suitable animal. Provided the teeth of the animal are sufficiently large and so positioned as to allow ready access to the pulp and periapex, experiments may be designed to simulate most situations encountered in the practice of endodontics. Various endodontic drugs and materials may be applied to vital pulps, and provided a sufficiently large number of animals are examined, the variation in tissue response may be assessed microscopically. Root canals, either infected or sterile, may be intentionally underfilled or overfilled with various materials and the reaction of the periapical tissues ascertained after extended observation periods.

There are, however, some intrinsic difficulties inherent in the use of animals for endodontic research. It is not always possible to produce experimentally the various pathological changes which affect human pulps requiring treatment. Relative tissue compatibility of drugs and materials may be investigated in animals by treating healthy exposed pulps, but the capacity of these materials to counteract pre-existing inflammation or degenerative change cannot be assessed. Again, it is a matter of speculation as to whether the data obtained from some animal research can be validly extrapolated to the human situation. The animal of choice is therefore one in which the tissue response bears a similarity to the human.

Various experimental animals have been used in the past, and it is probable that the tissue reaction in a member of the Primate suborder Anthropoidea would most closely approximate that in man. Large numbers of animals are required, however, and there are very real problems of cost, availability, handling and long term care with monkeys.

The difficulties of handling are minimised when using small animals such as rats and guinea pigs and probably for this reason more than any other, the rodent has been used extensively for some aspects of endodontic research. With these animals, however, there is not only the difficulty of access to the pulp chambers which hinders operative precision, but the marked healing capacity exhibited by the traumatised rodent pulp is not found in human teeth. In this regard, Kozlov and Messler[137] state that the amputated pulps of rodents tend to heal readily in a stereotyped manner with dentine bridge formation if the agent applied is non injurious and the fillings do not leak and are not lost. Again, the size and position of the teeth and the molar root anatomy limits the range of endodontic investigations which may be undertaken in the rodent. Erasquin and his co-workers[31,76,77,78,79,174] have devised a method for root canal treatment in the molar of the rat, but they appear to be the only investigators to have successfully utilised the animal for this aspect of experimental endodontics.

Among the larger animals suggested for endodontic research is the miniature pig[250] but it would seem to offer few advantages over other Eutheria such as the dog, which is readily available and which has been used for aspects of endodontic research by other workers. In 1932 Hill[120] examined experimentally induced apical granulomata in the dog and compared the findings with human material. Orban in 1933[182] investigated the action of paraformaldehyde on dog pulp and he formed the opinion that the periapical tissues in this animal are more sensitive to any kind of injury than in the human. This, he concluded is of significance, for endodontic experimentation which produces healing in the dog can be taken to

suggest acceptable therapy in man.

The outstanding work by Dixon and Rickert in 1938[66] on apical healing following root canal obturation was performed on the dog, and in 1956 Matsumiya and Kitamura[159] made significant observations on the fate of bacteria after treating experimentally infected dog root canals. Further studies on periapical healing following treatment of sterile or infected root canals in the dog have been reported by Kukidome in 1957[141], Murata in 1959[173], Nygaard Ostby in 1961[185], Seltzer, Bender et al in 1964[220] and Snyder in 1966[232].

In the present study, the broad range of investigations exemplifies the flexibility permitted in experimental design when using the dog for endodontic research. The experiments provide an insight into the reaction of dog pulp and periapical tissues to various endodontic procedures, and an attempt has been made to assess the validity of extrapolating findings in the dog to the human situation by reviewing the relevant literature on human studies.

It is not the object of this work definitively to investigate the range of tissue reaction to individual endodontic procedures. Rather, the aim of the thesis is to assess the suitability of the dog for experimental endodontics, and the selection of experiments described may serve as guidelines for future research using this animal.

MATERIALS AND METHODS

In this series of investigations 21 Alsation dogs were used, of which 18 survived the full term of the experiments. One animal died of anaesthetic overdose, another animal was lost due to Distemper and the third animal did not survive postoperative haemorrhage following a 'debarking' operation. The holding period averaged six months, but experimental periods ranged from two weeks to fourteen months.

Teeth used.

The 2nd, 3rd and 4th mandibular premolars were used in the investigations. The 2nd premolar is usually single rooted, but occasionally it is similar to the 3rd and 4th premolars, possessing a mesial and distal root, the canals of which are sufficiently wide to allow instrumentation to the apical region with conventional equipment. The crowns are blade like, and the pulp chamber passes from a prominent central horn to smaller mesial and distal horns before entering the root canals. It is possible to open into each canal individually, and different root canal treatments were frequently carried out in the mesial and distal root of a single tooth for the purpose of subsequent comparison of periapical response The mandibular premolars are not in contact with the maxillary teeth and any apical periodontitis which may supervene following operative procedures is not complicated by occlusal stress.

Fig. 1 page 6. The coronal pulp tissue in the mandibular premolar of the dog conforms to the blade like shape of the crown before passing into wide, straight mesial and distal root canals.

Fig. 2. Morphologically the pulp tissue is similar to that seen in a human tooth. The stroma of the pulp, the odontoblast layer and the predentine zone are seen in the photomicrograph.

Fig. 3. The main root canal breaks up into a series of fine channels at the cemento-dentinal junction. These apical ramifications are confined to cementum.

Fig.1 x12

Fig.2 x125

Fig.3 x25

The pulp tissue of the dog is morphologically similar to human pulp when examined microscopically but the anatomy of the root apex is distinctive. At the cemento-dentinal junction, which is situated at some distance from the actual apex, the main canal breaks up into a complexity of fine channels which radiate peripherally, and these canal branches are all confined to the cementum. Although such complexity is not seen in apices of human teeth, apical ramifications do occur[10,117,139,160] and the main canal often pursues a tortuous course to its apical termination, especially in posterior teeth. The impossibility of mechanically cleansing these ramifications and curvatures is accepted, and the marked complexity of the dog apex is regarded as an advantageous morphological feature when endeavouring experimentally to eradicate bacteria from these regions. Retention or restoration of vital tissue in these ramifications following various endodontic procedures is an indication of successful therapy.

The range of experimentation.

In order to compare the reaction of canine and human pulp, various endodontic materials and drugs were selected and applied to exposed dog pulps. The limited number of experiments with each material did not enable definitive assessment of the range of reaction which may occur in the dog, but sufficient information was usually obtained to permit comparison with what is known of human pulp reactions in similar circumstances.

Pulp exposures or pulpotomy wounds were treated with calcium hydroxide, the iodoform paste KRI, a corticosteroid/antibiotic cement and paste Ledermix, and the action of N_2 was compared with other paraformaldehyde materials such as Oxpara and Cysi Triopaste.

The reaction of the periapical tissues to root canal filling with various materials was also surveyed. Pulpectomy was followed by root filling to the cemento-dentinal junction in some cases, while in others, perforation of the apex permitted extrusion of materials into the periapical tissues. The reaction to zinc oxide

based root canal sealer (composed of precipitated silver 40% zinc oxide 60% mixed with a liquid made up of eugenol, resin and canada balsam) was compared with the periapical response to paraformaldehyde pastes and the resorbable paste KRI 3.

In some instances, experimentally infected canals were root filled with the above mentioned materials in an effort to induce healing of apical areas of reaction. Usually one of the two infected root canals in a tooth was left untreated as a control while in other cases differing root canal treatments were effected in each canal for the purpose of comparing the periapical response.

It was hoped that this range of experimentation would permit an insight into the reaction of dog periapical tissue to endodontic procedures carried out within and beyond the root canal and disclose areas for profitable research in the future.

Experimental infection of root canals and the production of apical areas of reaction.

In a number of cases, apical areas of reaction were stimulated by inoculating root canals, from which the pulp had been removed, with a 24 hour culture of alpha haemolytic streptococcus (strain 4) incubated in nutrient broth after transfer of a standard inoculum from a stock culture refrigerated in glucose broth containing $CaCO_3$. A paper point, size 5, was saturated in the suspension of bacteria, cut in half and the tapering portion inserted into the root canal. Streptococcus viridans, which is most frequently recovered from infected root canals[225] is considered ideal for the purpose, for it incites a low grade periapical response without danger of acute exacerbation.

Investigations were carried out by Mr. Schamshula of the Institute of Dental Research, Sydney,[206] analysing the bacterial counts from points impregnated in the above manner. The cut paper points were placed in 5 mls. of peptone water containing glass beads and the points were shredded in a vortex mixer for 3 minutes. Serial dilutions were made to 1/1000 and cultures and counts were made on mitis salivarius agar medium. Cultures plated out from

the 1/100 dilution were found to be countable. In six trials, the bacterial numbers ranged from 20×10^3 to 107×10^4. It is possible however, that the organisms located apically in the canal were the only ones remaining viable and capable of producing the reaction periapically and no further effort was made to standardise the inoculum.

The apical granulomata usually became radiographically discernible within 2-3 months but the extent of the radiolucent areas varied considerably even in the same animal where the same culture and inoculation technique had been used. Microscopic examination often revealed a marked difference in the intensity of reaction around control apices and there was variation in the extent to which bacteria proliferated and established themselves in tooth apices. Further investigations would be required to determine whether inoculation of identical numbers of bacteria would reduce this variability in response to any significant extent.

With regard to the granulomata produced, Hill[120] in 1932 found that they were morphologically and histologically comparable to those developed in the human. He noted that they were produced more quickly and easily in the dog than in humans possibly as a result of the greater number of apical foramina. Freeman[88] examined over 200 human granulomata and found at least 45% of these were devoid of any epithelial tissue. The incidence of epithelial proliferation in dog granulomata produced in the above manner was found to be considerably less in the present study.

Bacterial spread into apical ramifications from the main canal as well as into dentinal tubules was commonly noted (figs. 6,7.). Of particular interest however, was the dissemination of bacteria into cemental lacunae (figs. 9,10,11.). This was noted by Matsumiya and Kitamura[159] and Hayashi[114] and verified by the present writer as a commonly occurring event. The apical cementum is quite porous, possessing a network of fine channels which provide a pathway for the spread of bacteria into the lacunae often throughout the apical third of the root.

Although some earlier writers mentioned infected cementum in human teeth as a possibility[23], this phenomenon, as seen in the dog, does not seem to occur in the human apex. Nicholls[176] doubts that resorption of the root canal apex is due to invasion of lacunae and cemental canaliculi by micro-organisms, for he finds the pathway from the main canal to the lacunae in the human apex difficult to envisage. Pickard[189] considered the occurrence of infected cementum in the human improbable, and Shovelton[226] makes no mention of bacteria in apical cementum following his investigations into the distribution of bacteria in non vital root apices. The present writer sectioned 20 human apices with apical granulomata attached and found no evidence of cemental penetration by bacteria.

It is probable that the occurrence of infected cementum in the teeth of the dog makes healing of apical areas more of a problem than in humans due to the difficulty of eradicating bacteria from such protected sites.

Fig. 4. Three months after inoculating the canals of the lower right third premolar with streptococcus viridans, a radiographically discernible area of reaction has developed around the distal apex and to a lesser extent around the mesial root end.

Fig. 5. Low power photomicrography shows the apical granuloma distally while little response has occurred mesially. This variability in reaction to bacterial inoculation is common and difficult to explain.

Fig. 6,7.Spread of bacteria into the tubules from the main canal was a common observation.

Fig. 8. Granulomata have developed over the roots of the lower left third premolar (See figs. 32, 33 for higher power photomicrographs).

Figs. 9,10,11. Photomicrographs at various magnifications show the spread of bacteria into cemental lacunae around the apex of the mesial root of the tooth shown in fig. 8. Note the porous texture of the cementum especially at the cemento-dentinal junction permitting the spread of bacteria.

d m

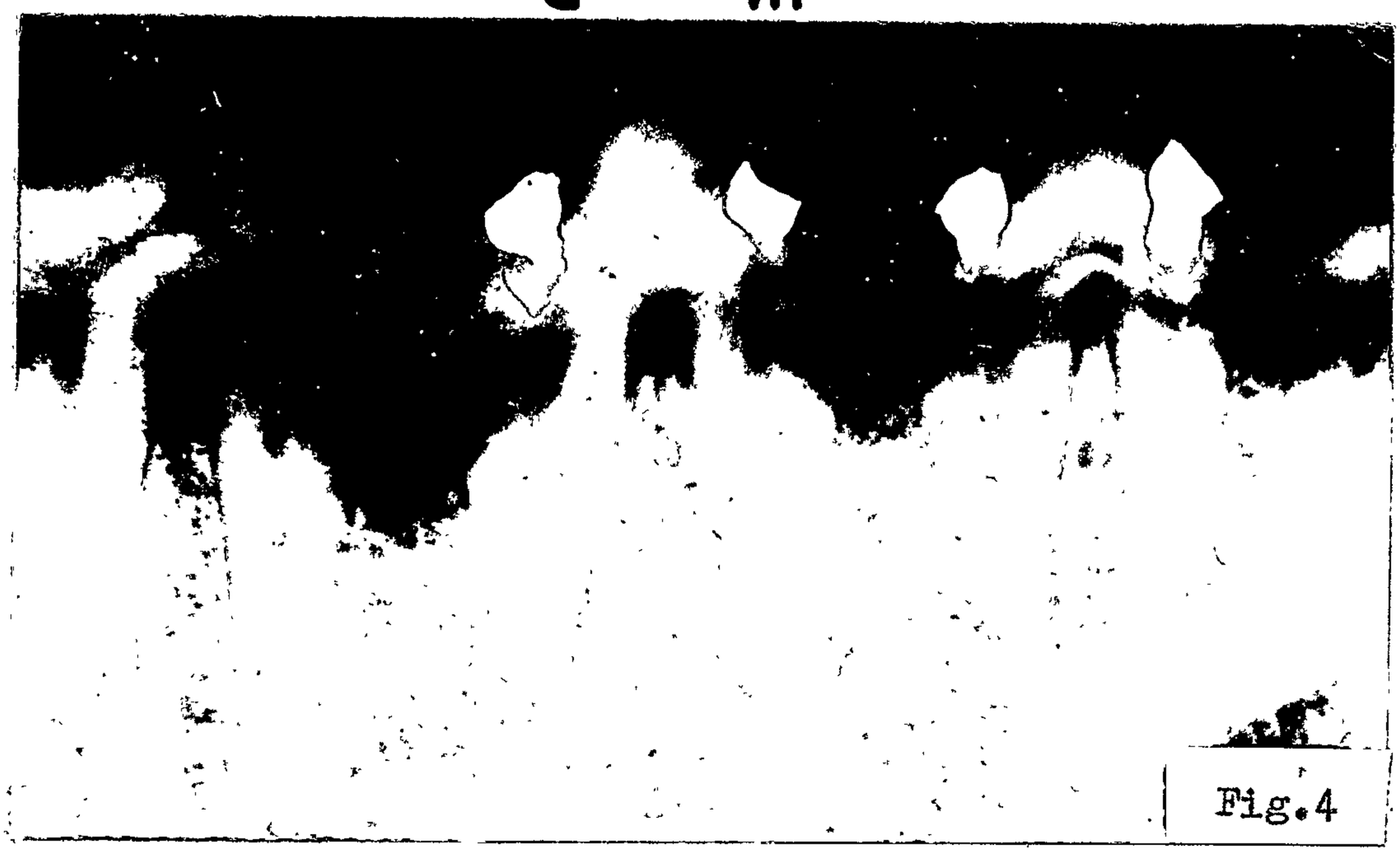


Fig.4

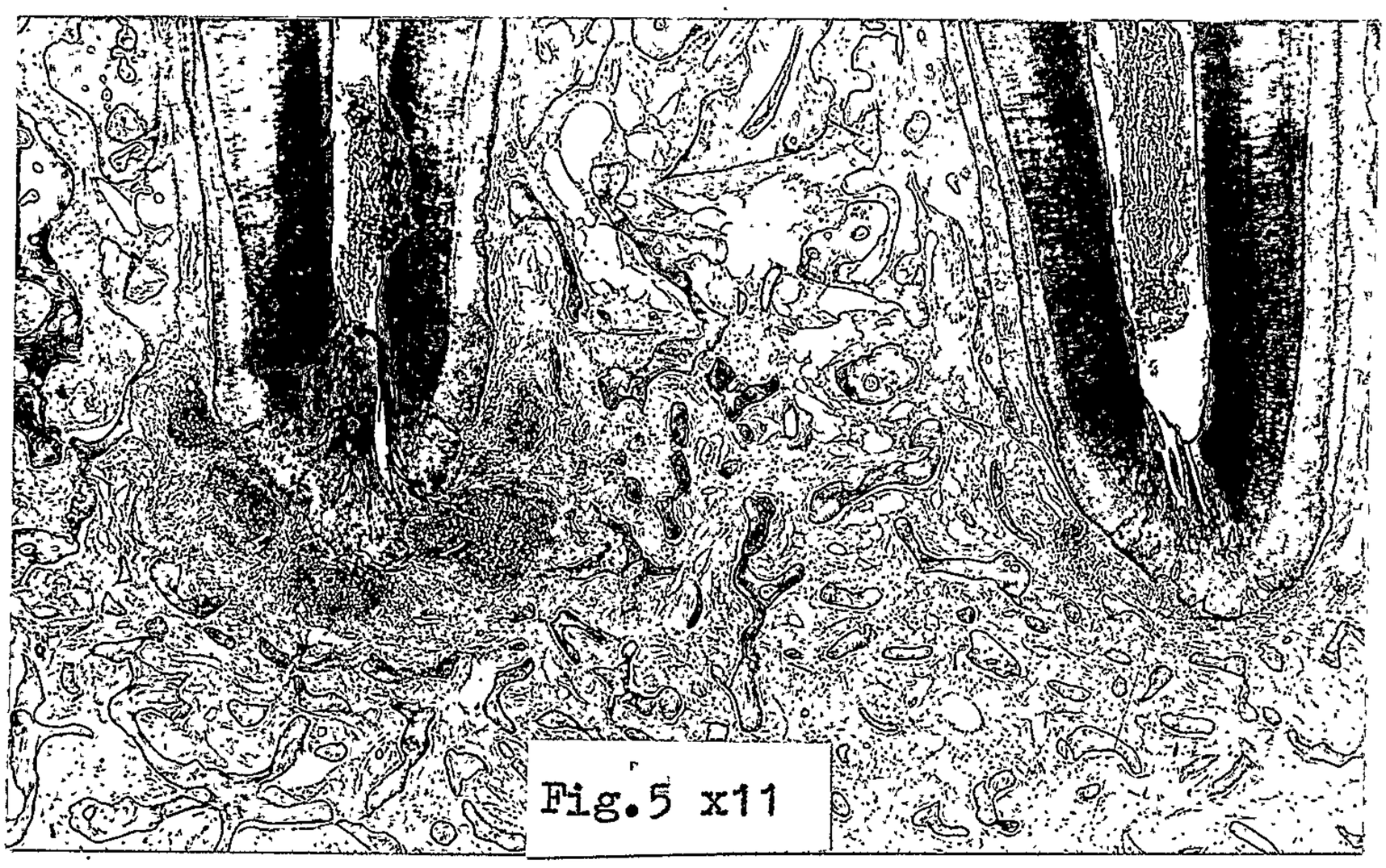


Fig.5 x11

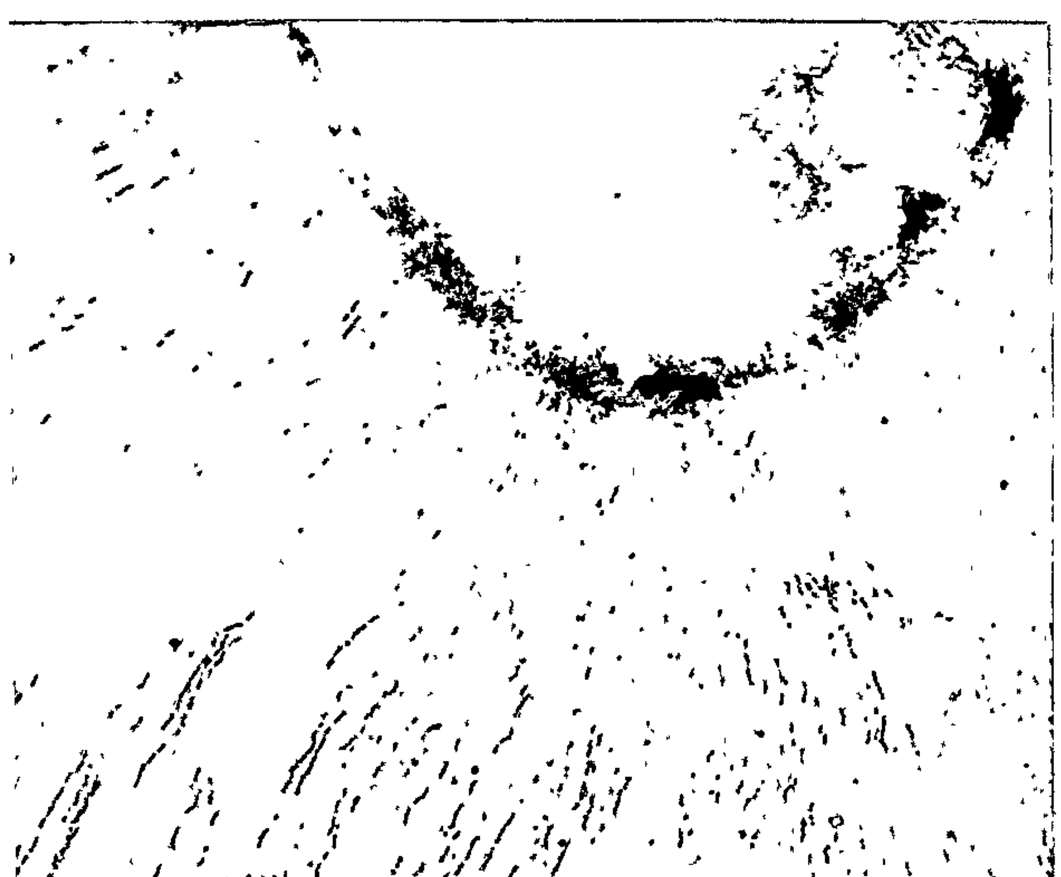


Fig.6 x125
GRAM

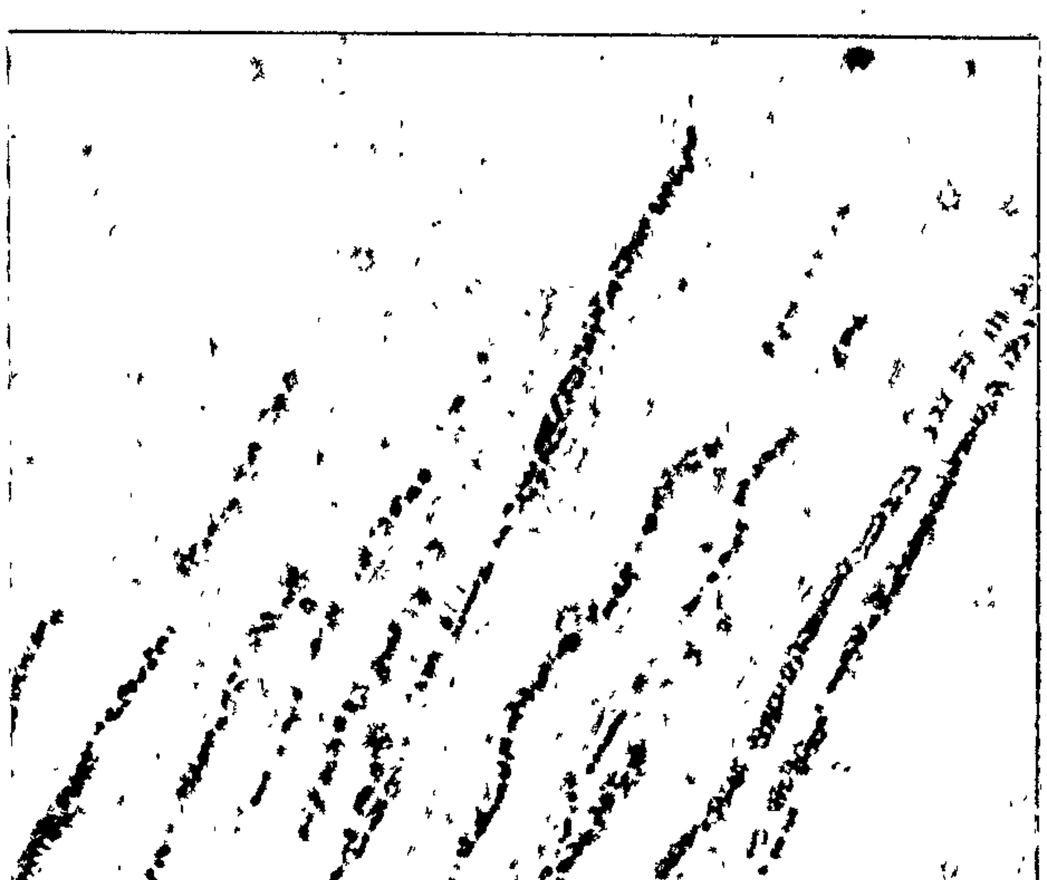


Fig.7 x560
GRAM.

m d

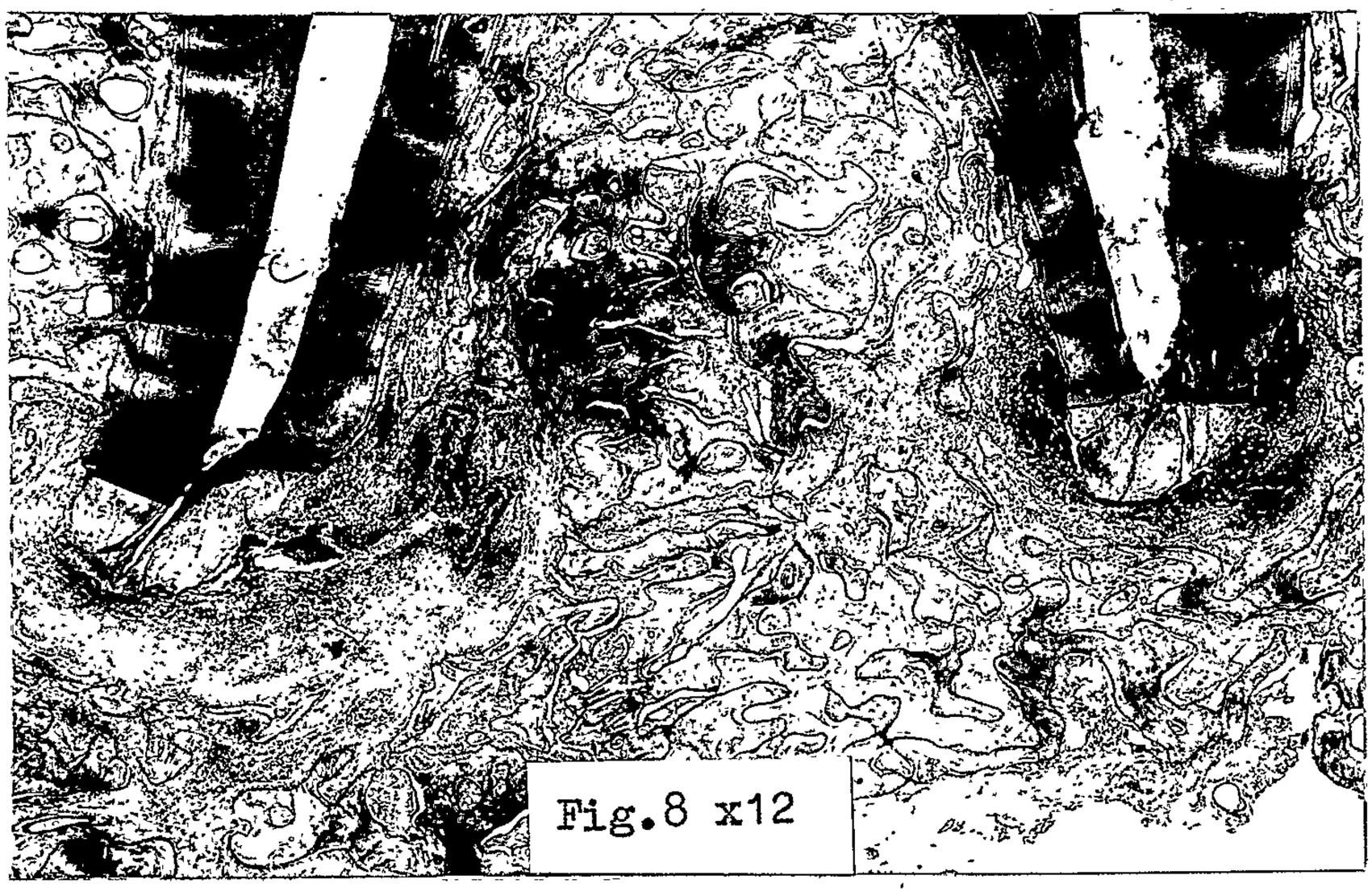


Fig.8 x12

Fig.9 x32
Gram

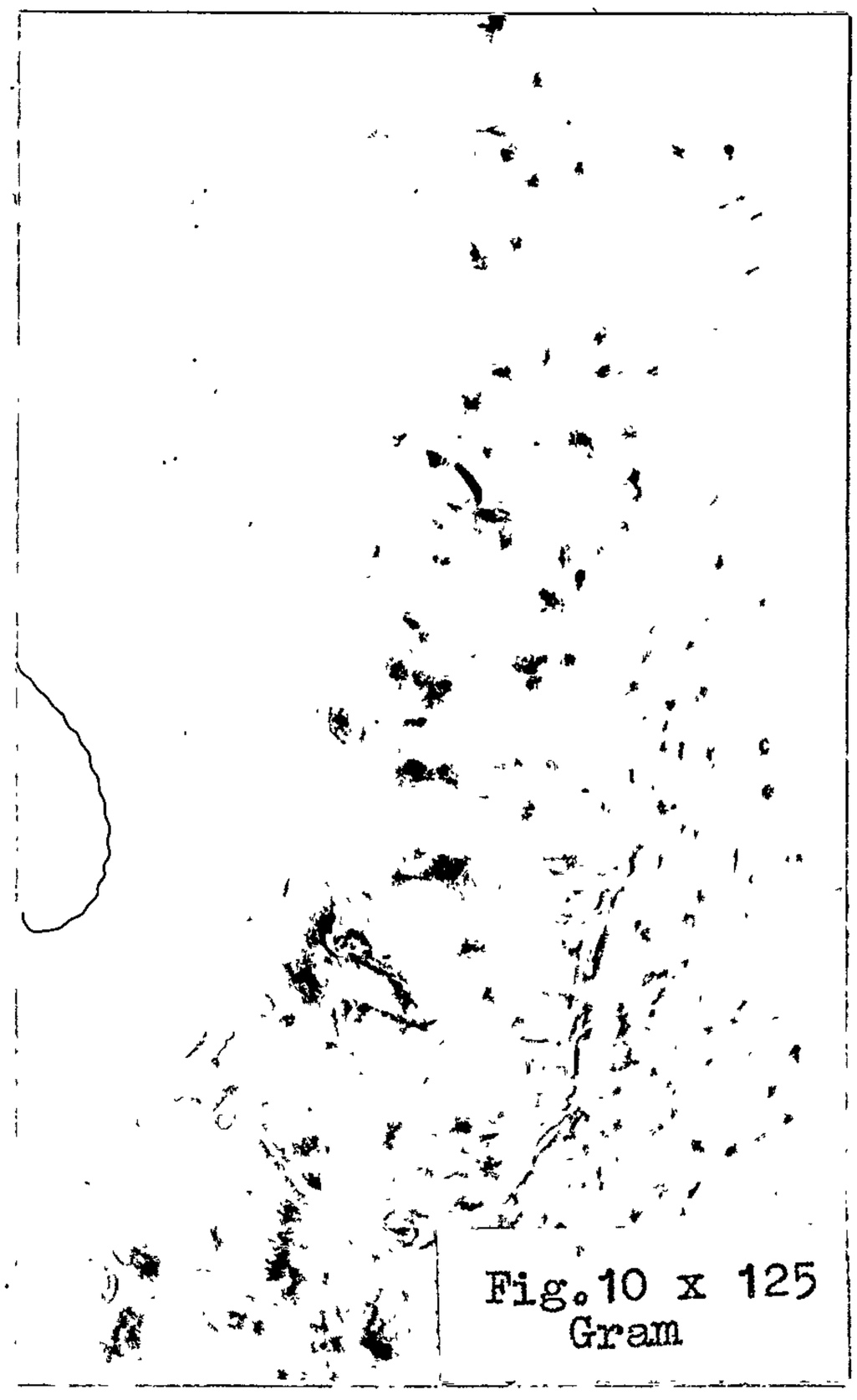


Fig.10 x 125
Gram

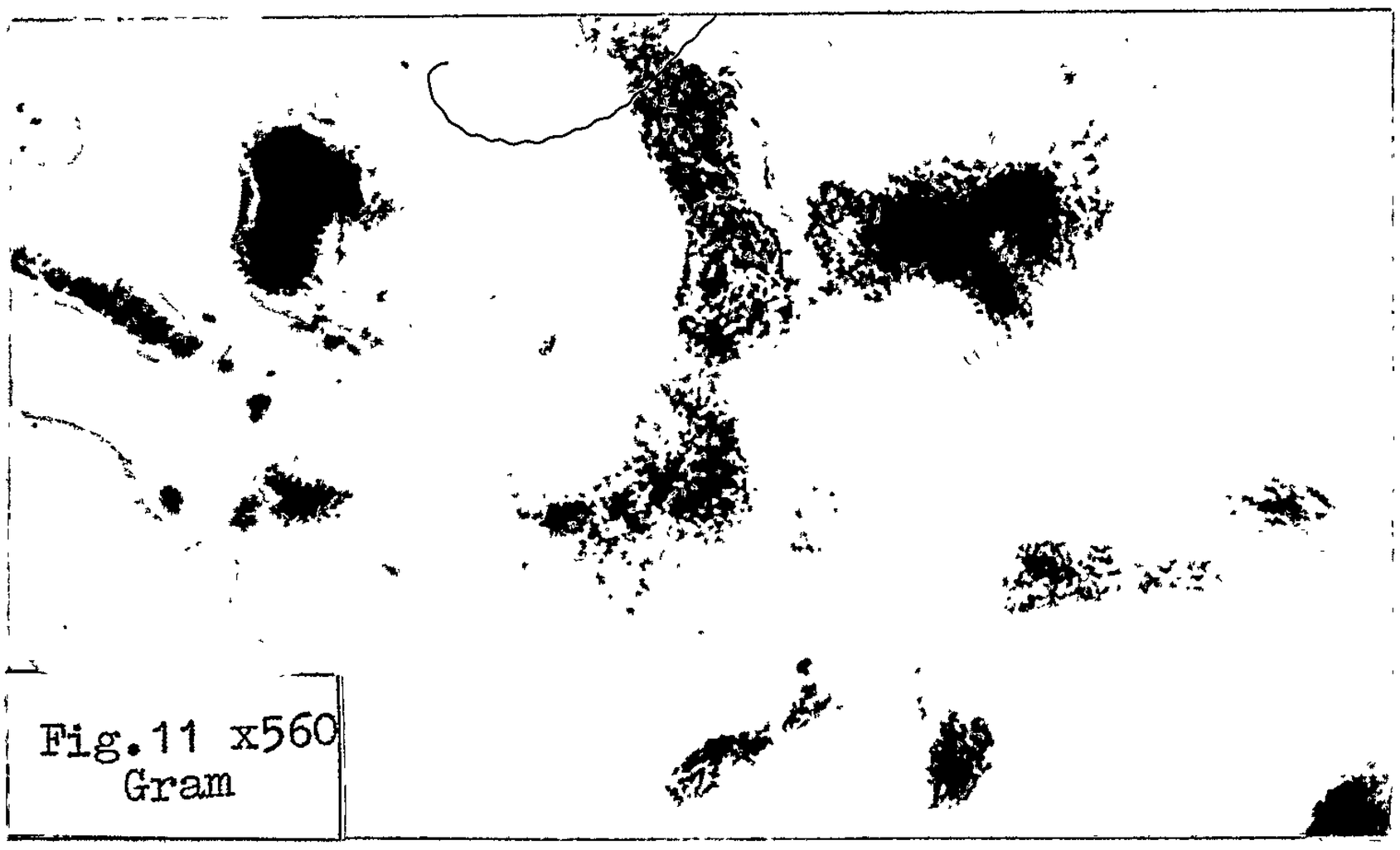


Fig.11 x560
Gram

Gram stain was used to locate Gram positive bacteria in sections of teeth. This procedure in itself did not identify the micro-organism stained as streptococcus viridens, but cultures taken from a selection of experimentally infected canals after granulomata had developed and prior to further treatment yielded a pure growth of alpha haemolytic streptococcus in each case. These tests were not carried out on the total series, and the possibility of subsequent accidental contamination by other micro-organisms cannot be entirely discounted, but the recovery of the streptococcus in pure culture in a selection of cases does indicate that the experimental technique employed militated against the introduction of extraneous infection. Gram positive forms which exhibited the morphology of bacteria in tissue were therefore provisionally identified as the progeny of the alpha haemolytic streptococcus originally inoculated. Fluorescent antibody staining technique would have been superior to Gram staining for specificity, but the development of this procedure was not pursued in this study.

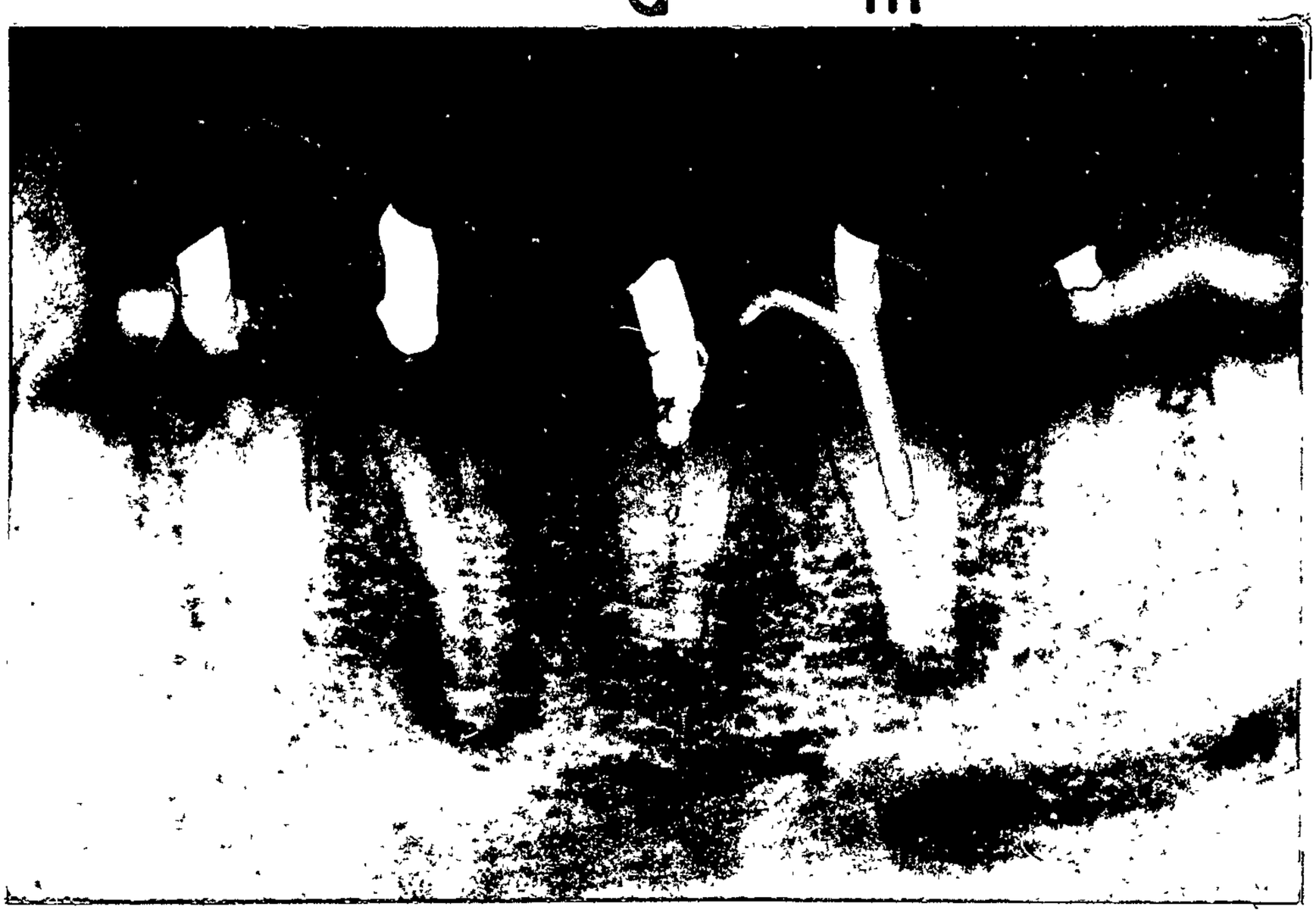
Failure to demonstrate bacteria by Gram staining did not conclusively indicate that a previously infected root canal had been sterilised by the endodontic treatment employed or by the tissue reaction. Alternate slides only were stained with Gram/ eosin, the remainder being preserved for tissue stains, and it is possible that a sparsely distributed residual bacterial population escaped observation. Surgical removal of the root apex followed by culturing would have provided a more reliable indication that bacteria had been eradicated, but this procedure necessarily precludes the histologic observation of periapical tissue reaction and was considered impractical. To summarise therefore, it was tentatively assumed that pre-existing infection had been controlled- if not completely eradicated following endodontic treatment - if Gram staining of alternate sections gave negative results and periapical inflammatory change was minimal or absent.

Although the practice of inoculating streptococcus viridans into canals whose apices were unperforated was adopted in the present investigations as a means of generating apical areas of reaction, some preliminary experiments indicated that more severe apical irritation resulted following initial perforation of the apices.

The mesial apex of the lower right third premolar (Fig. 11e) had been perforated, the canal flooded with a suspension of the streptococcus and partial filling effected with gutta percha. The radiograph was taken after 6 months and extensive bone resorption was seen to have occurred around the apex. The photomicrograph (Fig. 11b) depicted the ingrowth of inflammatory tissue into the canal lumen, and the cell population was predominantly of the polymorphonuclear variety (Fig. 11c). Gram positive bacteria were stained in the canal.

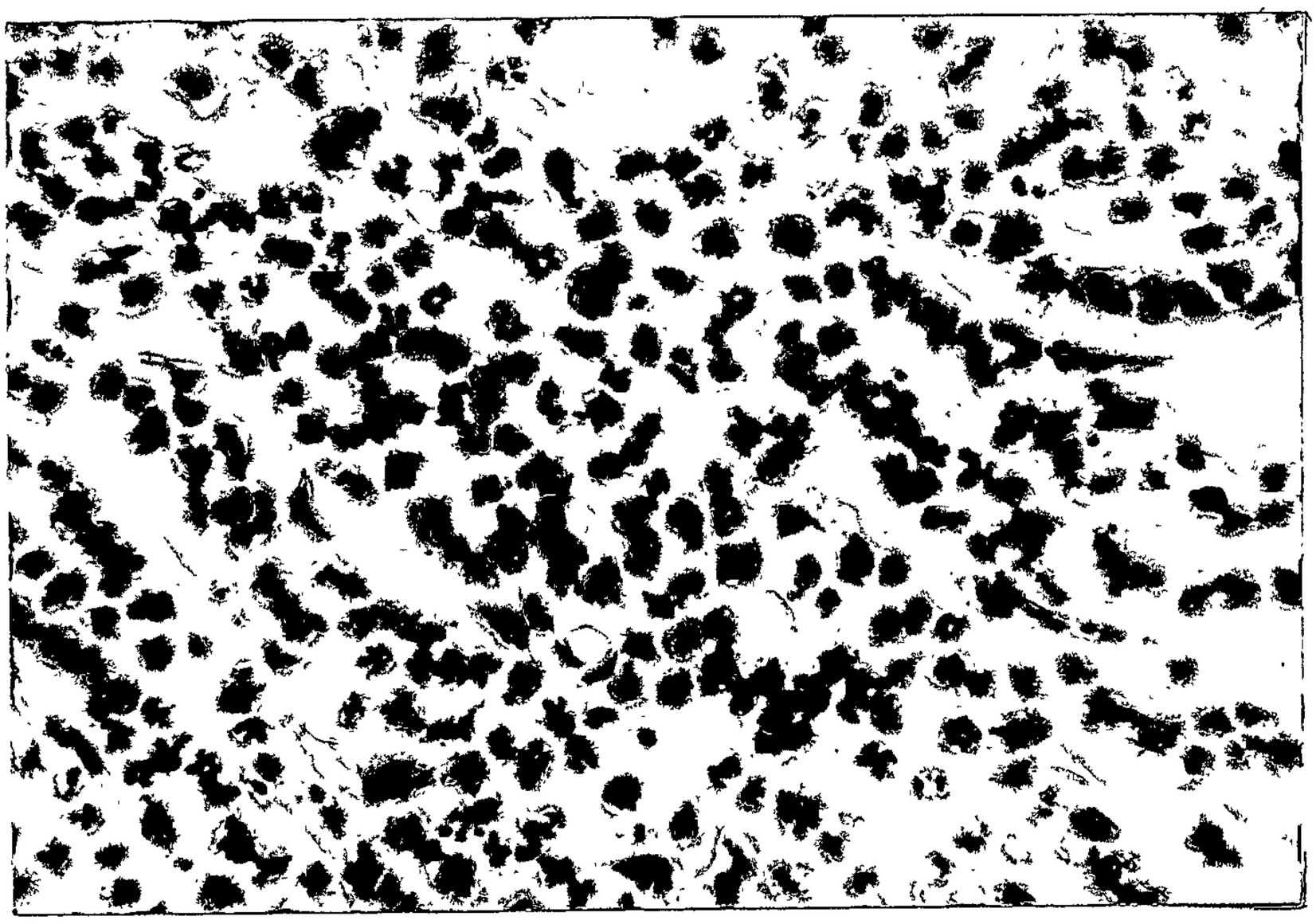
Paper points, impregnated with the streptococcus, were found to be intensely irritant when extruded through a perforated apex. Figure 11d shows a severe apical abscess which had developed one month after an experimentally infected point had been passed into the periapical region. The paper point apparently provided a nidus for bacterial growth for Gram stain detected large numbers of Gram positive bacterial forms on strands of the point in the apical tissue (Fig. 11e, b). The result of a similar experiment is shown in Figure 11f where an abscess had persisted for 6 months after a paper point impregnated with a suspension of streptococci had been inserted into the perforated canal. Inflammatory exudate with masses of polymorphonuclear leucocytes had seeped into the canal around the point, but bacteria still flourished in the interstices of the paper point in the depths of the root canal (Fig. 11g).

In one experiment (Figs. 11h-j) a sterile paper point appeared to be as irritant as an infected one when passed through the perforated apex in a lower right 4th premolar (Fig. 11h,s). Points of small size were selected so that they would work through the apices, and after sealing off the sterile point in the mesial canal, the distal canal received a point impregnated with streptococcus viridans. The radiograph taken after 3 months shows



11a

11b x44



11c x560

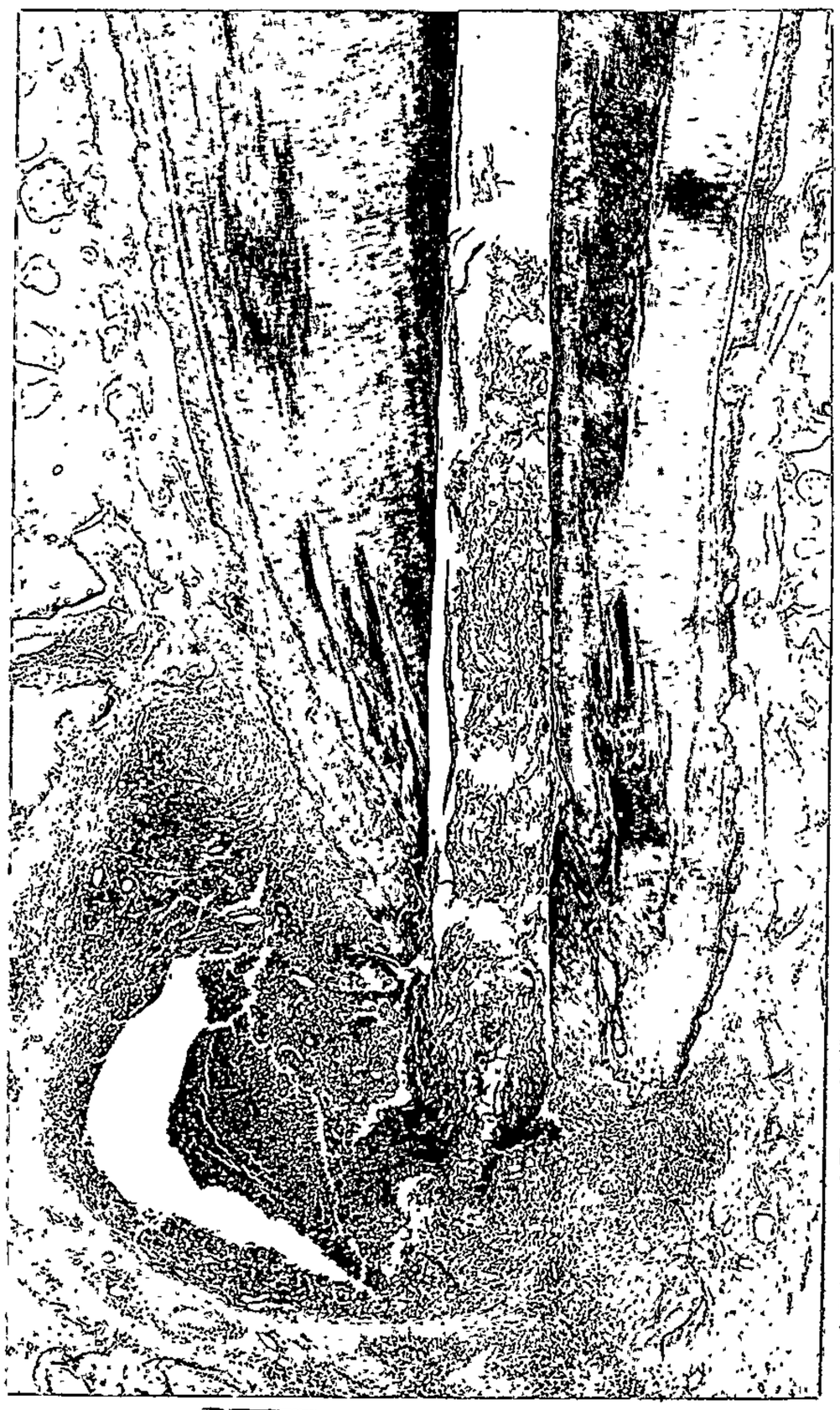


Fig. 11d x20.

Fig. 11e x560
Gram

Fig. 11f x20

Fig. 11g x560
Gram

Fig. 11h

Fig. 11i x10

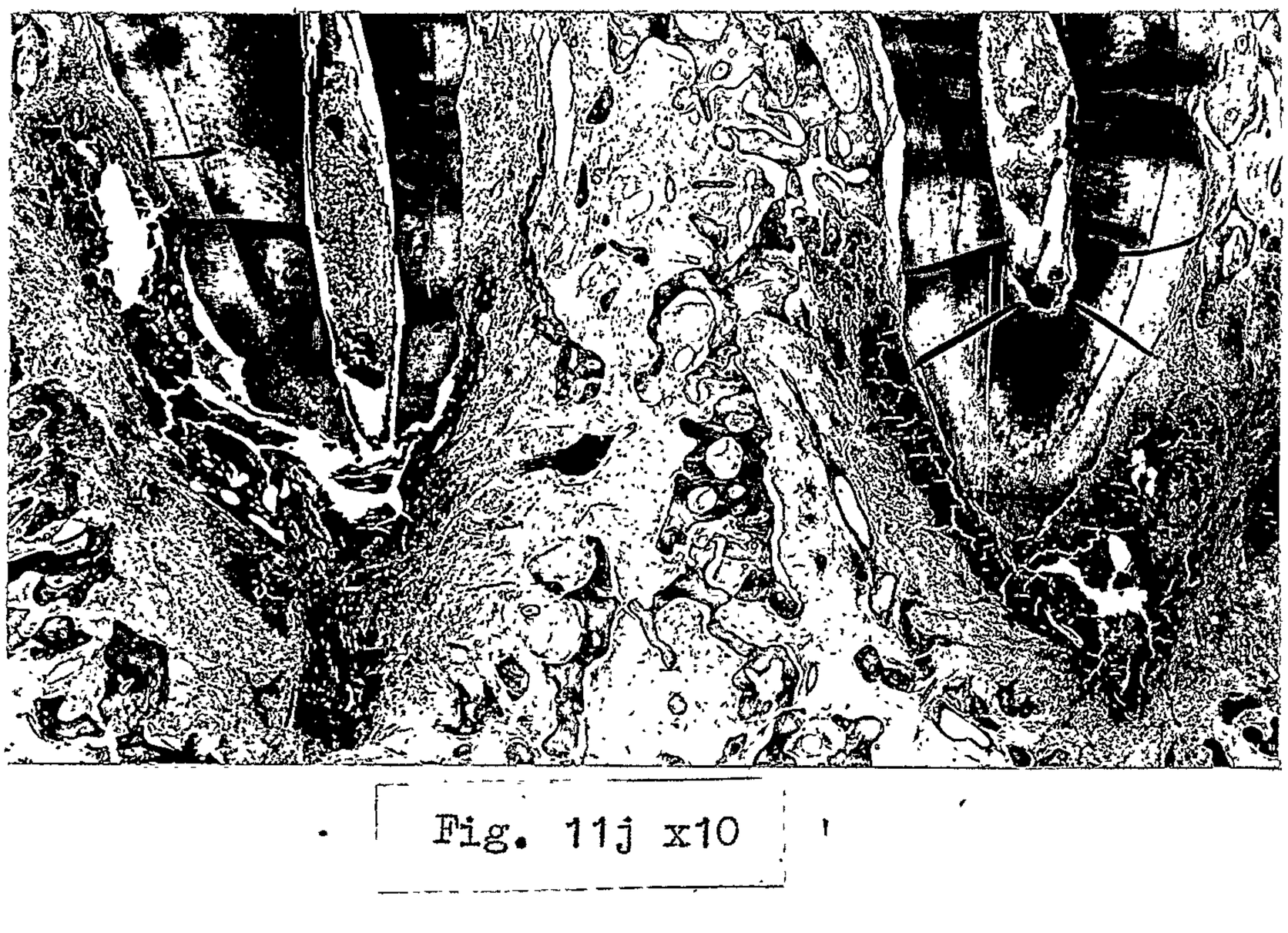


Fig. 11j x10

areas of reaction over both root apices and sections at two different levels show an abscess of some extent over each root. This illustrates the known clinical fact, that sterile paper points in contact with periapical tissue may cause severe irritation.

While relatively severe apical reactions may be rapidly produced by these methods after perforating root apices, consideration of the animal's well-being militated against further experimentation in this manner. It must be pointed out, however, that close observation of the animals which were subjected to these pilot experiments did not detect any apparent impairment in activity or disposition.

Surgical technique

In this section, consideration will be given to the maintenance of general anaesthesia, the preservation of an aseptic method of working, and the radiographic technique.

In quiet animals, the intravenous administration of a tranquilising agent such as NORTRAN (Trifluomeprazine) is an effective premedicant, and may be followed immediately by injection of a medium acting barbiturate such as SAGATAL (Pentobarbitone Sodium Veterinary). Initial premedication as described results in loss of corneal and pedal reflexes after half the basal dose of barbiturate has been administered, and this margin of safety allows the injection of supplementary doses for maintenance of surgical anaesthesia over prolonged operating periods.

For reasons of practical availability, the dorsal metatarsal vein is preferred for venipuncture where it crosses the lateral aspect of the hind leg, although haematoma formation in this area may necessitate the administration of maintenance doses via the cephalic vein of the foreleg.

In many cases, however, the restraint necessary for animals weighing from 50 to 140 lbs. prohibits intravenous premedication, and initial intramuscular injection of an analgesic agent must be administered. Intramuscular morphine is used in difficult cases and it permits the administration of intravenous barbiturate in the shortest time, but the necessity of using dosages up to 2 grains

leads to side effects of vomiting, bowel evacuation and respiratory depression. When time permits, and when handling active animals which are required to return for several visits, intramuscular premedication with THEMALON VETERINARY (Diethylthiambuten hydrochloride) is preferred. This morphine substitute requires up to one hour for full tranquilising effect, but the absence of side effects is of importance. This premedication is followed by intravenous SAGATAL.

An aseptic method of working is facilitated by application of rubber dam and the provision of a sterile and a working surface[7]. The teeth are thoroughly cleaned with wheel brushes and the rubber dam perforated to pass over the 2nd, 3rd and 4th mandibular premolars. The dam is readily applied to these teeth, and the constriction at the cemento enamel junction permits stabilisation with clamps and ligatures (Fig. 13). The teeth are then scrubbed with a solution of camphorated para mono chlor phenol.

The sterile surface is in the form of a trolley on which are placed autoclaved instruments and disposable materials. These instruments are transferred as required to a working area on the operating table to which they are returned after use. A further bench area is required on which medicaments and pastes may be prepared for transfer to the working surface (Fig. 12).

This procedure follows the method advocated and used by the writer[7] in a dental surgery when practicing endodontics, and while bacteriological tests were not employed at all stages of the experimental work to verify that strict asepsis was similarly maintained, it is unlikely that the risk of contamination was any greater when working with the dog.

Radiographs were obtained using a Stanford machine 90/30 operating at 75 Kv and 30 ma. with an object distance of 15 inches. A focusing light box was used to reduce scatter. On the living animal, the periapical film was held in place by the operator using lead lined gloves. In consequence of the anatomy of the dog mandible, the film angulation changed considerably from the 2nd to

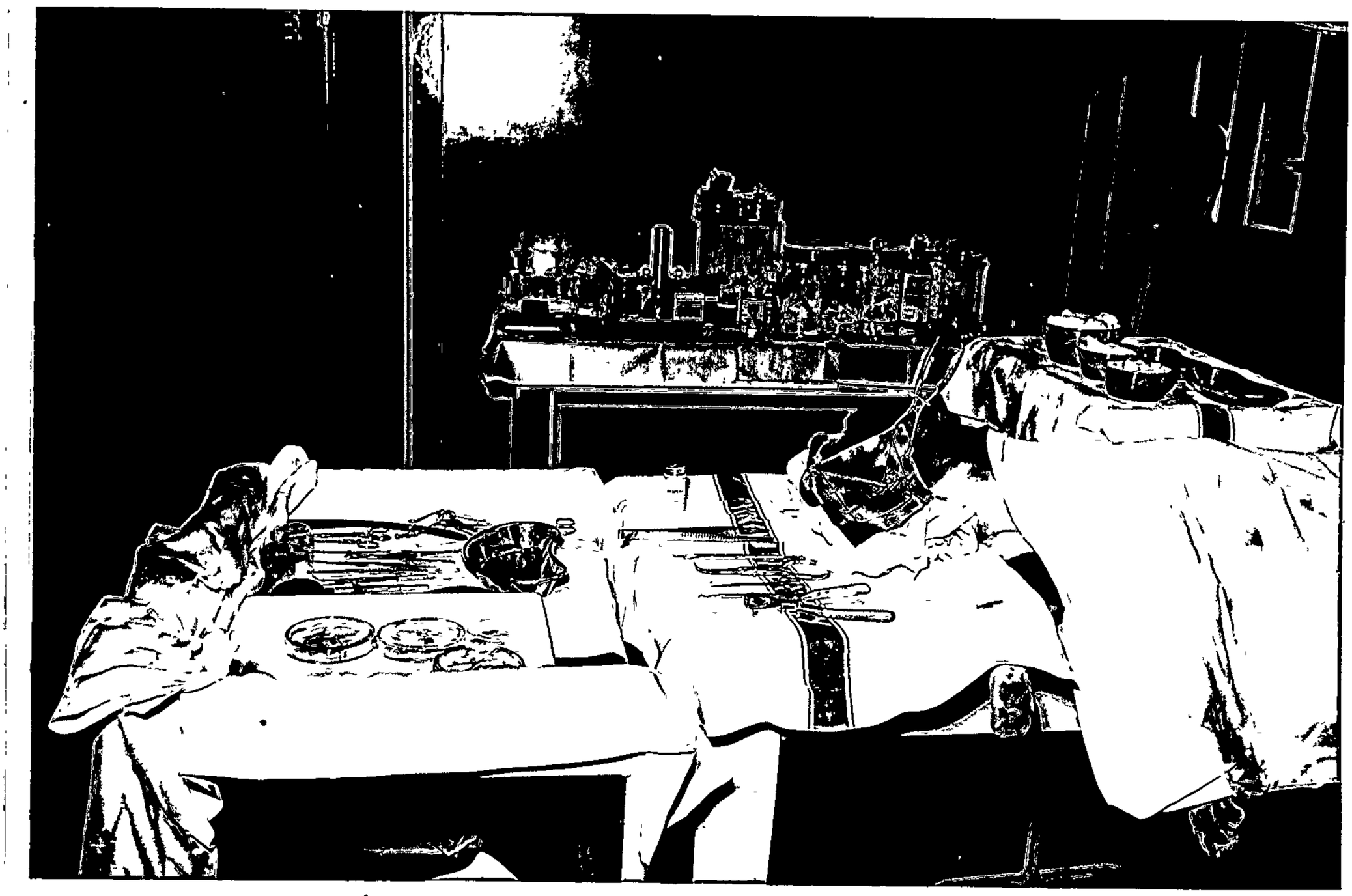


Fig.12

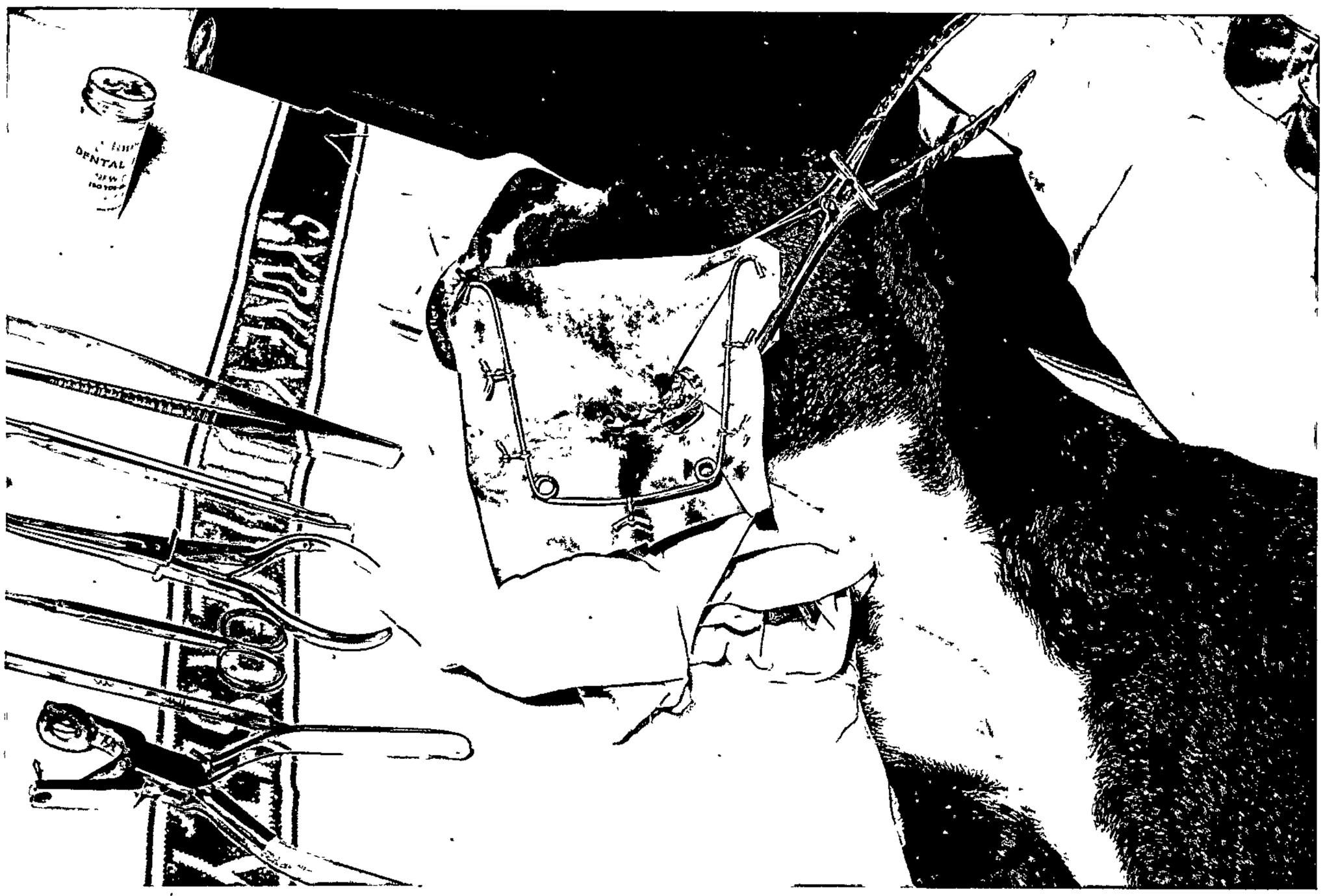


Fig.13

the 4th premolar region. In view of this fact, and the necessity for leaving dam and clamps in position during exposure of the film, it was not possible to achieve standardised angulation and this shortcoming had to be borne in mind when comparing films in an effort to interpret periapical change[17].

Notes on histological technique.

The animals were perfused with 10% formol saline (ph 7) via the common carotid artery. Gravity pressure was used in the region of 140mm of mercury, and perfusion continued until the fluid returning from the external jugular vein of the opposite side was clear. The mandibles were dissected free, separated at the symphysis and individual tooth blocks obtained using a fine toothed saw. Initial cuts were made through the cortex and radiographs taken to check whether the line of division was true.

Decalcification was effected with formic acid 7%, the solution being changed daily and the progress of decalcification checked radiographically[25]. Superior staining characteristics were gained using lactic acid, but the extensive time required for decalcification of the large blocks limited the use of this agent.

The blocks were trimmed to as small a size as possible after clearing, for at this stage the roots were discernible through the semi translucent bone. When pulp histology was required, the block was sectioned at mid root level with a razor blade to allow maximum penetration of embedding material into the root canals.

The block size was usually found to be excessive for successful serial sectioning following paraffin embedding, and the celloidin technique did not permit cutting at a desirable thinness. A double embedding technique was therefore used, using Peterfi's method of celloidin impregnation followed by embedding in a paraffin/beeswax mixture[41]. After the embedded blocks had been orientated and trimmed to the critical level on a rotary microtome, the block was soaked overnight in a solution of 10% glycerine in 60% alcohol. The introduction of this step combined with the frequent cooling of the block with ice allowed ribbons of serial sections to be recovered at thicknesses of 8 - 12 microns.

Prior to mounting, the glass slides were flooded with an adhesive solution composed of 0.5% gelatine in glycerine, a procedure which prevented fragmentation of the section when subjected to drying temperatures.

Unless otherwise specified, all the sections shown were stained with haematoxylin and eosin.

SECTION 1

Healing following vital pulpectomy and root filling.

It is the purpose of this section to compare the reparative processes which occur apically in the human and the dog following root filling of non infected canals. The preliminary review of the literature will also consider the tissue reaction to overfilling with zinc oxide based root canal sealer and gutta percha.

The process of healing in human teeth following root canal filling is the subject of numerous reports by investigators, among whom are Davis[62], Grove[102], Blayney[23,24,25,26,27,28], Skillen[230], Hatton[77,78,79], Moen[166], Coolidge[44,45,46,47,48], Grossman[100] and Kronfeld[139].

Following pulp removal and root filling, an inflammatory reaction occurs at the site of amputation. Sinai and co-workers[22] carried out pulp extirpations in rhesus monkeys and humans, leaving an apical pulp stump. They confirmed that an acute inflammatory response occurred in the remaining pulp and in the periodontal membrane opposite the apical foramen and this lasted up to one month following pulp severance. Some resorption of cementum and bone occurred. These teeth were not root filled and it is of interest to note that a proportion of the apical pulps in the animals nevertheless healed.

After this period of inflammation, macrophages appear and phagocytose injured and dead tissue. Upon a fibrin framework, fibroblasts proliferate and lay down scar tissue. On the surface of the root canal near the apical foramen, an ingrowth of connective tissue may occur from the periodontal membrane followed by resorption of dentine and deposition of secondary cementum. Only occasionally does complete obliteration of the apical root canal occur, for a thin central canal is usually retained in the cementum leading to the apex. The tissues of the periodontal membrane take care of unfilled lateral branches and apical ramifications either by retaining vital connections with tissue contained therein or by forming cementum which may obliterate those canals. Then the cementum on the root surface is continuous over the former openings

of the ramifications.

While much is known concerning the healing process following root filling to the dentino-cemental junction in the human, further investigations are required into the reparative processes which occur following root filling to various levels, including the periapical response to overfilling.

In a clinical survey, Seltzer, Bender and Turkenkopf[219] reported that overfilling was definitely less successful in inducing repair than either underfilling or filling flush with the apex. They stated that overfilling causes irritation which hinders the process of repair, and referred to histologic studies which apparently showed the periapical tissues around extruded particles of cement and silver points to be in a state of severe inflammation. Grahnen and Hansson[95] observed 763 root filled teeth after 4 years and found that roots which had been filled with moderate or large excess had a significantly higher failure frequency than roots with little or no excess. Indeed, underfilling seemed to be preferable, and these results agree with Strindberg[239].

There is therefore a need for investigations into the tissue reaction following extrusion of root filling materials into the periapical region. Histologic examination of the cellular response following embedding of root filling materials into subcutaneous tissue and muscle has been carried out in various animals, but information derived from overfilling of root canals in a suitable animal would appear to be of greater value. The two most commonly used root filling materials are gutta percha and zinc oxide based root sealing cement, and it is appropriate in this review to study reports dealing with tissue response to these agents.

Gutta percha appears to cause little irritation. Hunter[123] in 1957 studied five implants of gutta percha in the tibia of guinea pigs and found after 6 months that the material was surrounded by a thin capsule of connective tissue with no evidence of cellular disturbance. He concluded that gutta percha was quite compatible with adjacent tissue whether it be bone, fat or fibrous

tissue. Following intramuscular implantation of gutta percha in rats, Boulger[33] observed a thick connective tissue capsule with no surrounding inflammation. Mitchell[163] in a similar investigation reported in 1959 that the reaction around the implanted gutta percha was mild after 4 weeks. Feldman[81] in 1962 placed this material in rat mandibles and found, after three months, that the implants were surrounded by cell-rich connective tissue capsules. In one case, bone was in direct contact with the gutta percha. Blayney[25,26] however, warned that it is advisable to underfill a canal with gutta percha, for he observed a case of overfilling where the extruded gutta percha point was surrounded by a rarefied area. He considers that extruded gutta percha causes pressure atrophy which reduces the resistance of the tissue to infection. Histologic support for this theory has not been forthcoming however. Oatby[185] confirmed the findings of other investigators and reported that gutta percha used to fill a canal was frequently found in direct contact with vital tissue, and when extruded into the periapex, it was surrounded by a fibrous capsule.

In one of the few available histologic studies on overfilling of root canals in either humans or animals, Muruzabal et al[174,78] reported on the effects of overfilling the mesial root of the first mandibular molar in the rat. Gutta percha points alone were soon surrounded by a connective tissue capsule. Extrusion of zinc oxide and eugenol cement, however, invoked a heavy infiltration of leucocytes within 24 hours and these were mainly polymorphonuclear cells. Five to ten days later, macrophages appeared and then fibroblasts commenced capsule formation, but macrophages persisted and slowly resorbed the extruded material. When the zinc oxide and eugenol cement came into contact with alveolar bone, necrosis occurred. This fact, together with the observation of intense tissue reaction in the periodontal membrane following extrusion of the material from the root canal, led these investigators to the conclusion that zinc oxide and eugenol is highly toxic to periapical tissues. It must be pointed out, however, that the inflammatory reaction was transient, and after 30 days fibrous encapsulation

had occurred.

Rowe[202] inserted various root filling materials into the root canals of cats' teeth and observed the effect on the periapical tissue after periods extending from two to twenty weeks. No attempt was made to perforate the root apices and extrude the materials into the periapex. Up to two weeks after removal of the pulp, results were similar irrespective of the root filling material employed. All showed considerable inflammatory reaction in the periapical tissues with infiltration of polymorphs, plasma cells and lymphocytes. Root filling with zinc oxide and eugenol gave disappointing results, for in six teeth, inflammation persisted in the periapical tissues after 16 weeks. When mixed with 15% barium sulphate and olive oil, however, there was some improvement.

Dixon and Rickert[65] found that zinc oxide based root sealer excited only very slight chronic productive inflammation when placed under rabbit skin; a surrounding wall of fibrous tissue was lightly infiltrated with lymphocytes and a few plasma cells. Hunter[123] found that a similar root canal sealer caused some fibrosis and a foreign body reaction with sustained phagocytosis when implanted in guinea pig tibia.

Seltzer et al[220] in their experiments with dog root canals used a zinc oxide based paste containing silver, and reported some 'violent' reactions after overfilling with this material. All their experiments, however, appear to have been carried out on infected root canals and it is possible that the reactions observed were generated by bacteria.

It is apparent that further studies on the effect of zinc oxide and eugenol based cements on periapical tissues are required

Apart from investigations into the effects of overfilling, further research is required in order to elucidate the reparative processes which occur following underfilling of root canals. While root filling to the cemento-dentinal junction is generally regarded as the ideal level, it does seem that repair of considerable magnitude will occur in cases of partial fillings provided there is no bacterial contamination. (Hatton [110]). Barker and Lockett[12] examined several imperfectly root filled teeth and found extensive

repair by soft and hard tissue even though the canals were grossly underfilled or lacked peripheral seal in the apical canal. Barker, Hession and Lockett[8], discussing another case of healing where vital lacunar cementum had filled the apical third of the canal in an inadequately root filled premolar, came to the conclusion that the level of obturation is not as critical as some clinicians maintain, at least following vital extirpation.

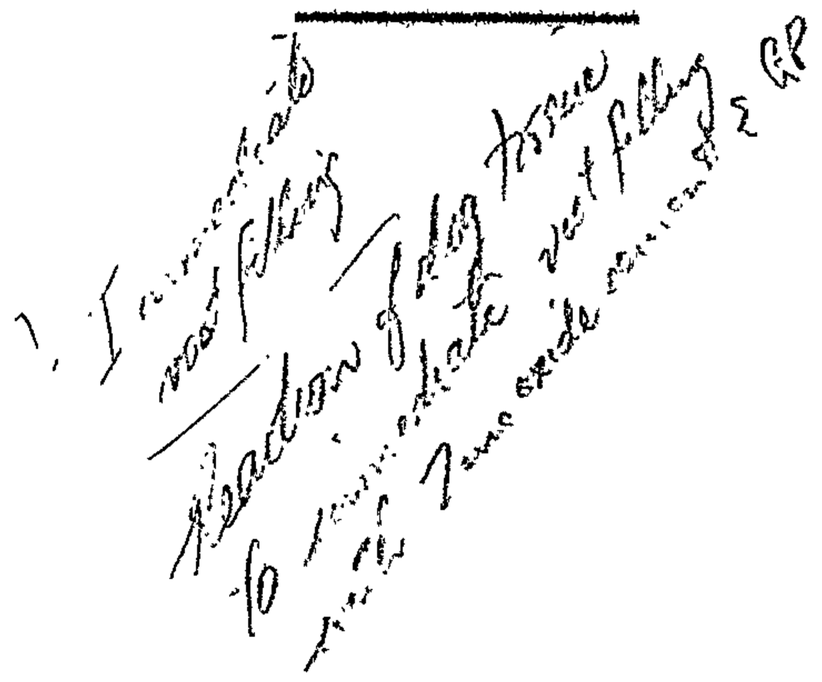
When a canal is obturated short of the apex, vital pulp tissue may be preserved in the unfilled apical portion, and if this retains its connections with the periapical tissue, some elaboration of dentine may occur. This was shown by Sekine, Machida and Imanishi in their experiments with partial pulpectomy[216].

More frequently however, the pulp is severed at the dentino-cemental junction and blood clot pervades the area. Organisation of this clot and phagocytosis or pulp residue results in an ingrowth of tissue from the periodontal membrane which may then form hard tissue, usually cementum. This may completely occlude the apical canal or merely line the walls. Some resorption of the dentine walls commonly occurs prior to the deposition of calcific tissue within the canal.

Ostby[185] undertook valuable investigations into the role of the blood clot in the apical portion of the canal following root canal therapy. In human teeth, bleeding into the canal was stimulated by lacerating the periapical tissues and then partial root filling was performed. Dogs were also used. Teeth were extracted, the apices cut off and the pulps removed, following which they were reimplanted and root filled in the cervical portion of the root canal only. Examination after periods extending up to 3½ years showed that in dogs, vital tissue extended 300u to 5mm into the canal. A similar situation occurred in the human tooth, the blood clot being organised by granulation tissue following which transformation into fibrous connective tissue occurred. There seemed to be an upper limit to the extent of the organisation process into the canal, and beyond the level of tissue ingrowth a space existed to the level of the root filling.

It appears, then, that if vital pulpectomy and root sealing can be effected in the coronal two thirds of the canal, healing in a large proportion of cases may be expected. Fish[85] favours immediate root canal filling in cases of vital exposure and states that provided no organisms have been carried into the apical canals, it makes little difference whether the canals are well filled or not, provided they are effectively sealed off from the mouth. He considers that it is more important to fill the cervical 2/3rds of the canal expeditiously than to risk carrying infection into the apices by prolonged instrumentation.

The following four experiments illustrate the suitability of the mandibular premolars in the dog for root canal filling investigations.



Experimental results.

Experiment 1. Figs. 14 - 17, page 30

In the lower right 4th premolar (fig.14,x) immediate root filling was effected to the cemento-dentinal junction in both roots following pulpectomy and canal enlargement with reamers and files. The root filling comprised gutta percha points cemented in place with a sealer composed of zinc oxide 60%, precipitated silver 40% mixed with a liquid made of eugenol, resin and canada balsam. Henceforth this material will be referred to as 'root canal sealer'. The apical ramifications in the cementum commence some distance from the actual apex, and the root filling therefore appeared underextended on the radiograph.

After 6 months, a radiograph showed an intact lamina dura around each root apex, and the healthy state of the periodontium was illustrated in a low power photomicrograph of the distal apex (fig. 15). Vital tissue persisted in the apical ramifications. Below the root filling lay a mass of dentine debris and apical to this was a plug of cementum (fig.16a). Whether this has been elaborated after root filling or whether it existed prior to operative procedures is impossible to say, but high power photomicrography (fig.17) suggested that cementocytes continued to reside in the lacunae and the vitality of the tissue had not been impaired. Remnants of pulp tissue remained adjacent to the cementum.

Experiment 2. Figs. 18 - 22, page 31

Following pulpectomy and canal enlargement, the mesial root canal of the lower left third premolar was immediately root filled to the cemento-dentinal junction. Distally the apex was perforated with a beutelrok drill and the root canal sealer was extruded into the periapical tissues together with a gutta percha point.

After 14 months the radiograph (fig. 18) showed the mesial apex circumscribed by a well defined lamina dura, and distally the absence of any radiolucency suggested that the paste and the gutta percha point had been well tolerated by the apical tissues.

Sections of the distal apex revealed the paste and gutta percha point

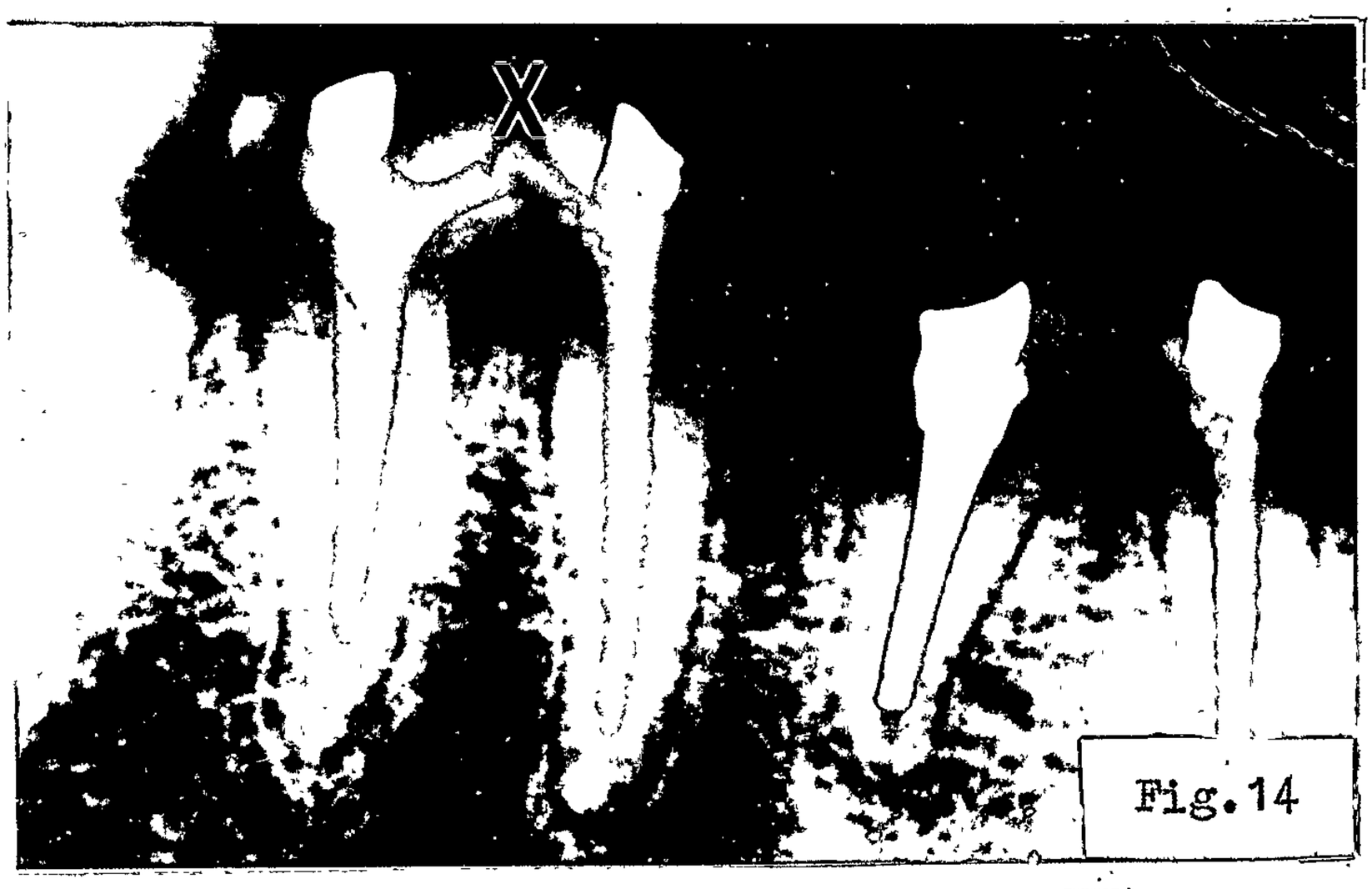


Fig.14

Fig 15 x32

Fig 16 x125

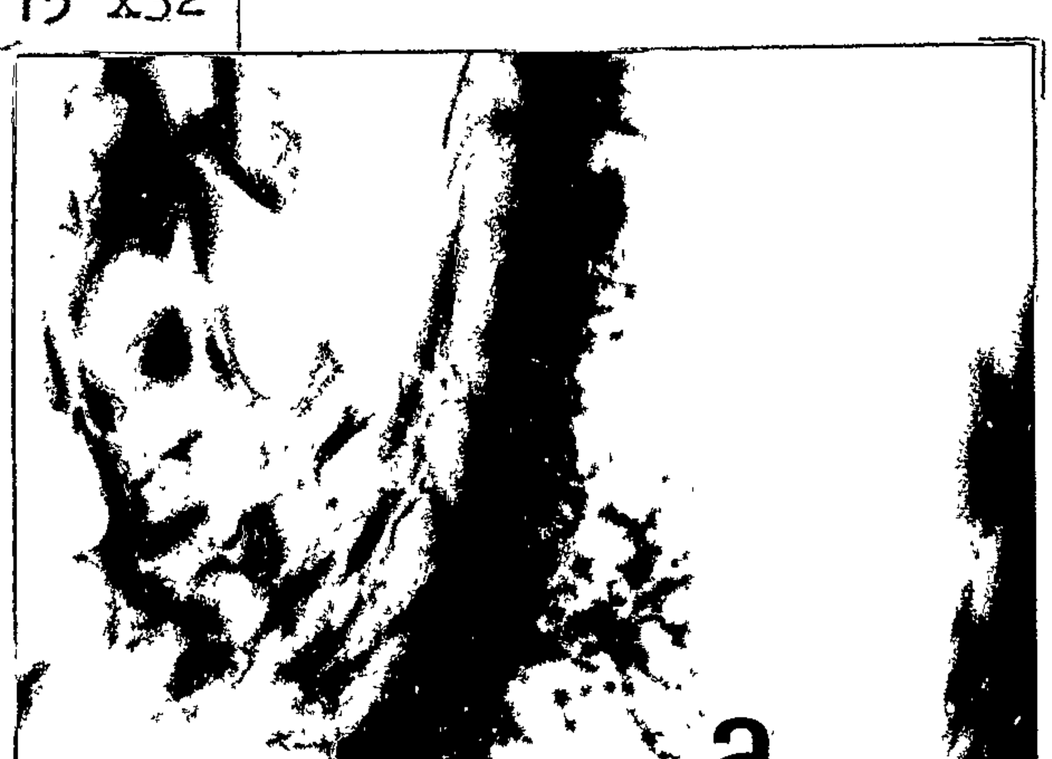


Fig.17 x560

Fig.18

Fig.19 x20

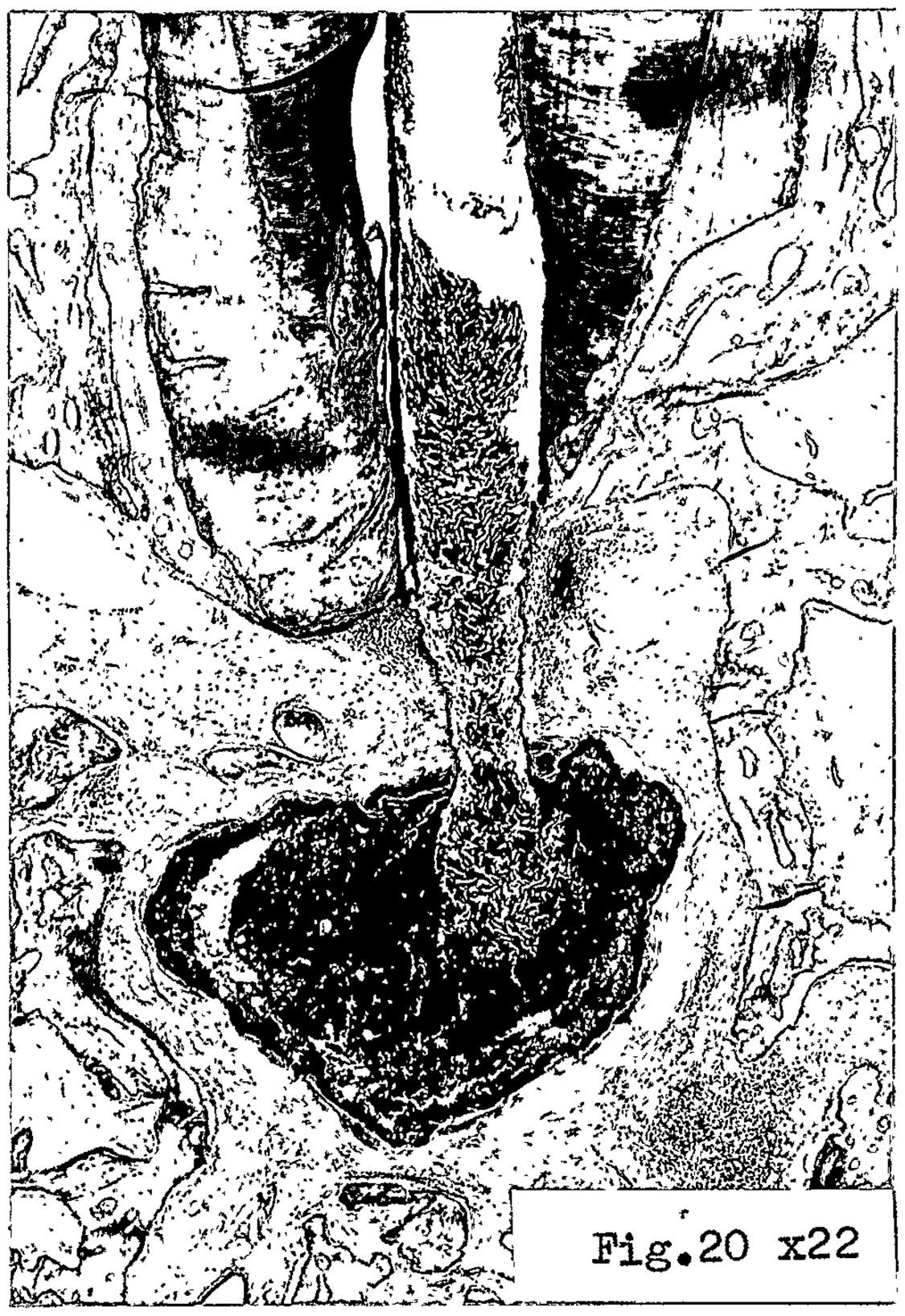


Fig.20 x22

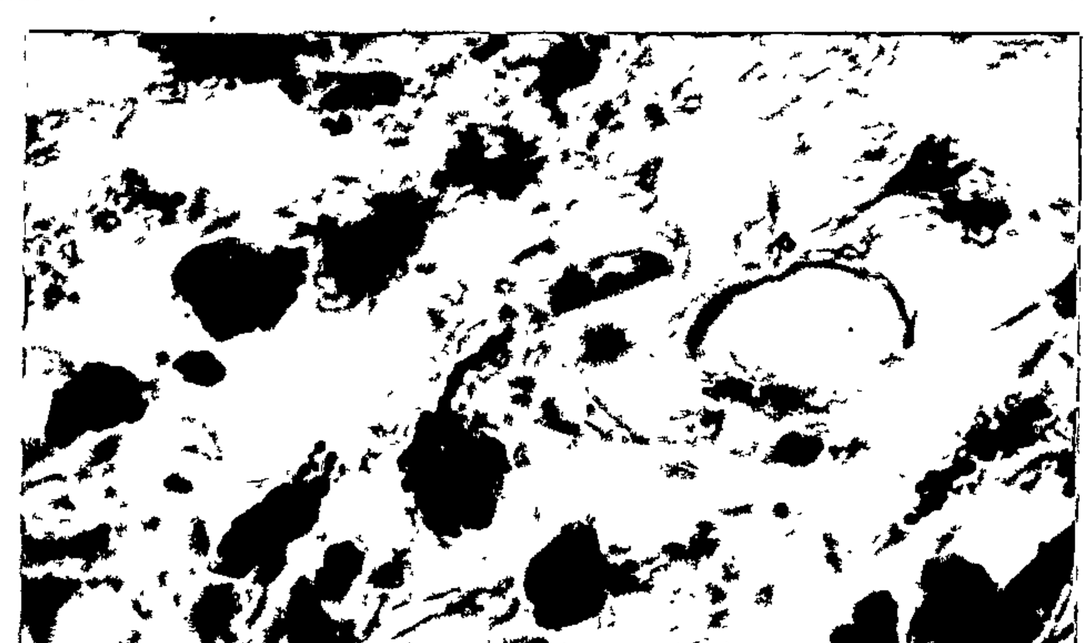


Fig.21 x560

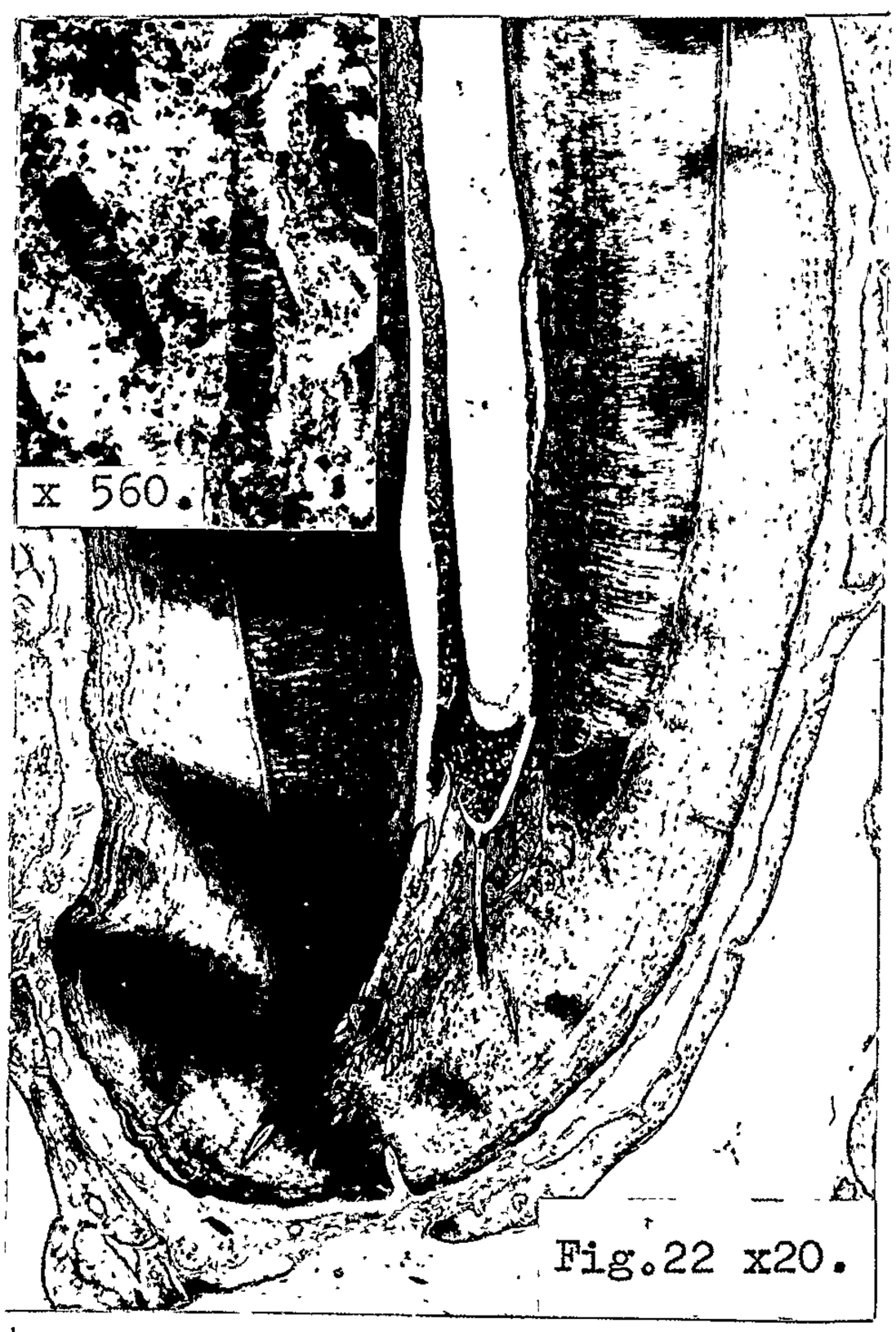


Fig.22 x20.

in the apical tissues (figs. 19.20). There was virtually a complete absence of inflammatory reaction and a thick sleeve of connective tissue encased the paste. Trabeculae of bone were in close proximity with the extruded material and these appeared vital. In some locations groups of macrophages were seen filled with granules of paste (fig.21). There was no discernible alteration in the amount of extruded material seen in the radiographs taken immediately postoperatively and after 14 months, and the removal of the root canal sealer from the tissues by macrophages must be a very slow process. The perforated apex had undergone some resorption and remodelling.

In Fig. 22 the mesial canal is seen to contain a large amount of dentine debris (high power inset, fig.22) alongside where the gutta percha point was inserted. This indicates the need for thorough irrigation during canal enlargement. The apical ramifications possessed normal tissue and the periapex was free of inflammation.

Experiment 3. Figs. 23 - 25, page 33

Immediate root filling to the cemento-dentinal junction had been carried out in the mesial root of the lower right 3rd premolar (u) while the distal apex was perforated and a gutta percha point with a small amount of root canal sealer had been passed into the periapical tissues. The radiograph taken immediately following root filling and that taken 12 months later (fig.23) showed that the amount of paste extruded had not diminished.

The lamina dura around the mesial apex appeared intact on the radiograph and this was confirmed on the low power photomicrograph (fig.24). The distal gutta percha point had impinged on the neuro-vascular bundle in the inferior dental canal but there was no inflammatory reaction (fig.25). Some paste was noticed around the gutta percha, and fibrous tissue condensation had occurred around both the paste and the extruded point.

Experiment 4. Figs. 26 - 29, page 35

Both root apices of the lower right 4th premolar were perforated and KRI paste was extruded into the periapex from the mesial canal. The results of this experiment are described on page 66. Distally, root canal sealer was conveyed into the

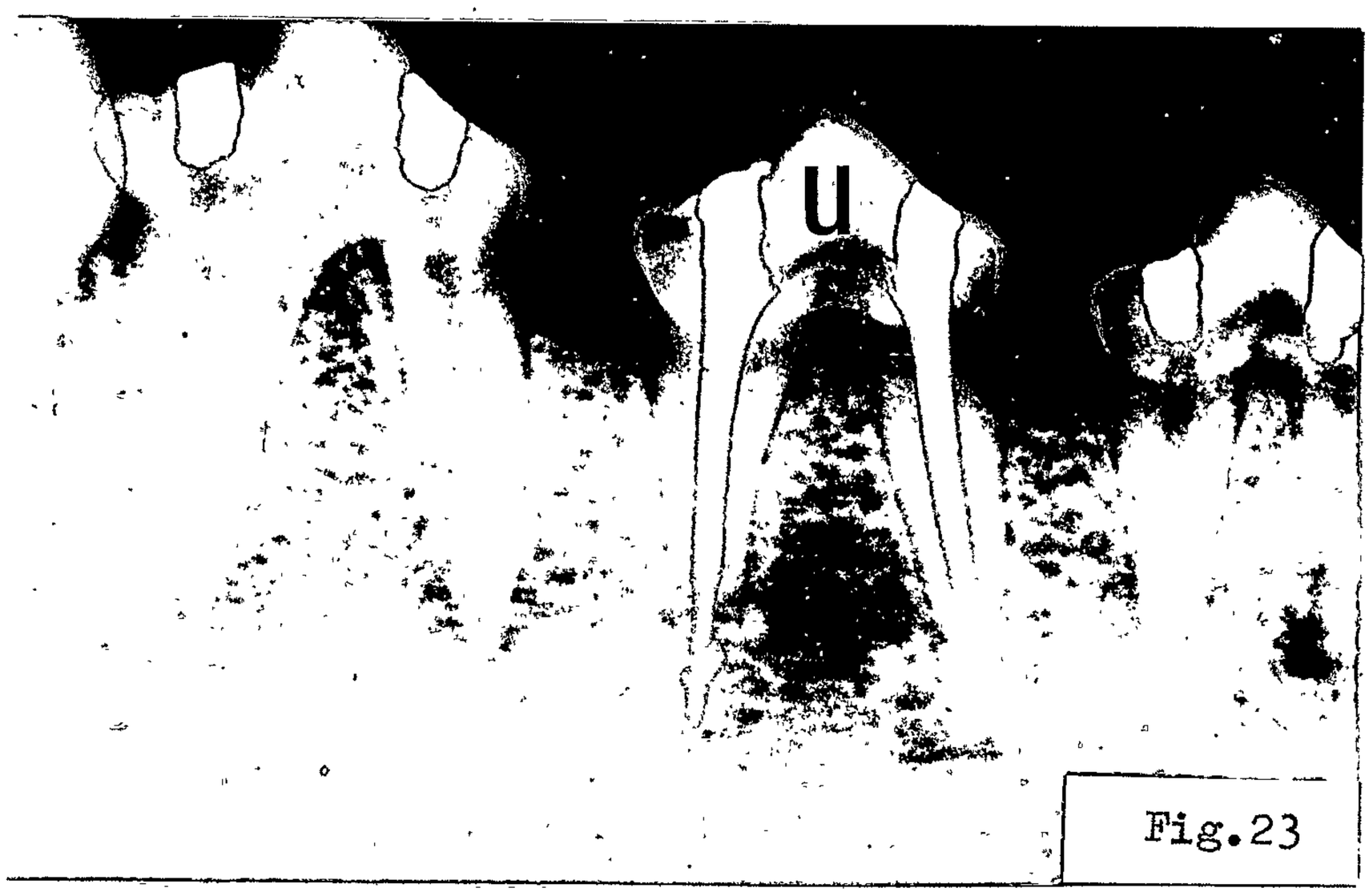


Fig.23

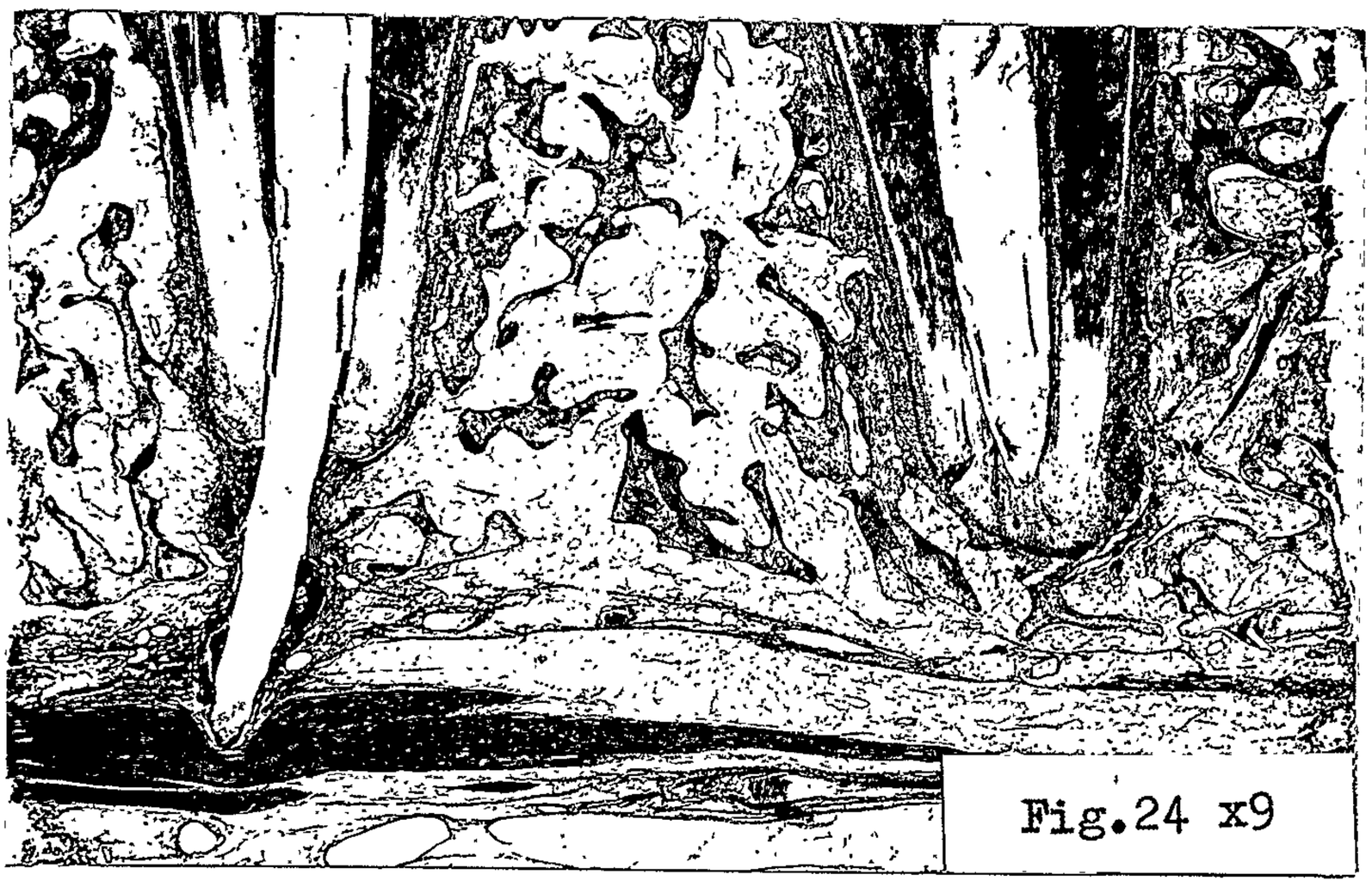


Fig.24 x9

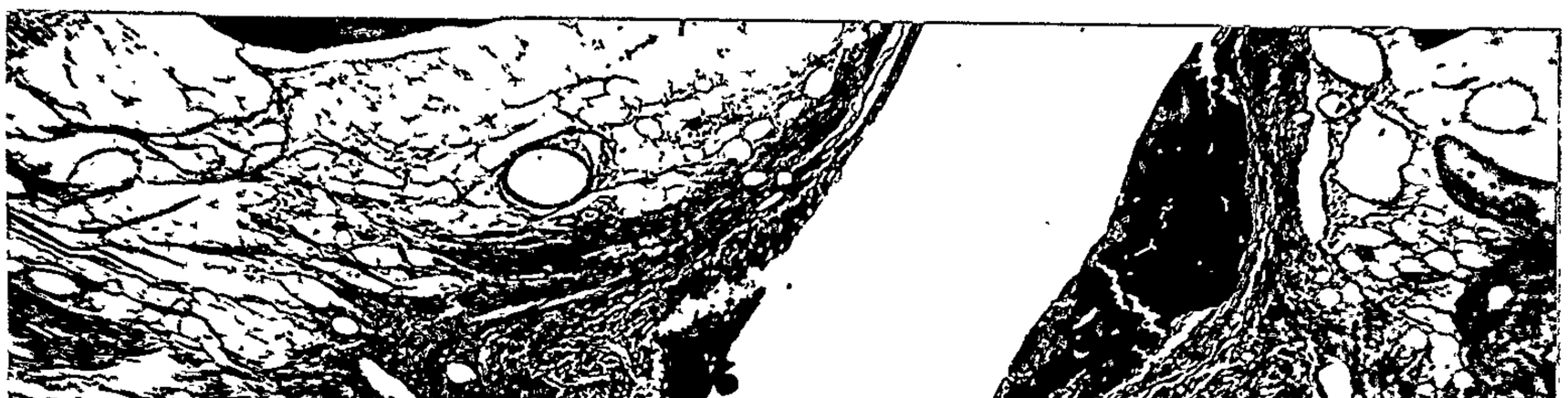


Fig.25 x32

periapical tissues by means of a Lentulo spiral instrument (fig.26). This experiment was allowed to continue only 2 weeks and in that time no resorption of root canal sealer was shown on the radiographs.

In the low power photomicrographs (fig.27), the root canal sealer was seen lying free in the tissue and it had aroused little reaction. Some areas showed slight round cell infiltration, but mainly the paste had excited a connective tissue walling off. In Fig. 29, a trabecula of bone with a layer of active osteoblasts was seen in very close proximity to the paste, separated from it by loose connective tissue which contained few inflammatory cells. Vital tissue was present in some apical ramifications.

Following perforation of an apex, a commonly observed reaction was resorption of the functionless part of the root tip, often with anchylosis to adjoining bone. Such an event had occurred in the position marked 'e' in Fig. 27. A high power photomicrograph of the anchylosed cementum is shown in Fig. 28.

Fig.26

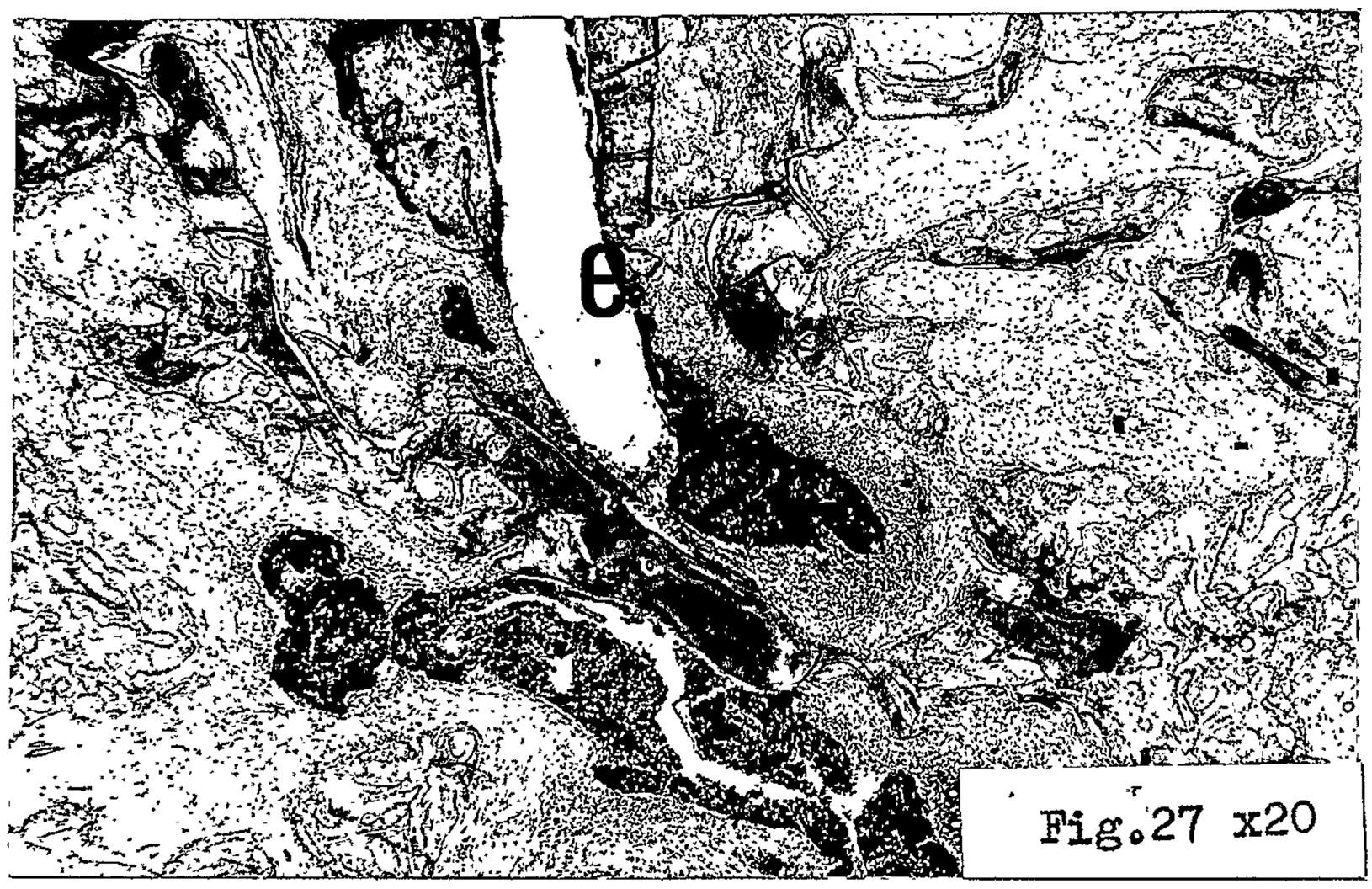


Fig.27 x20

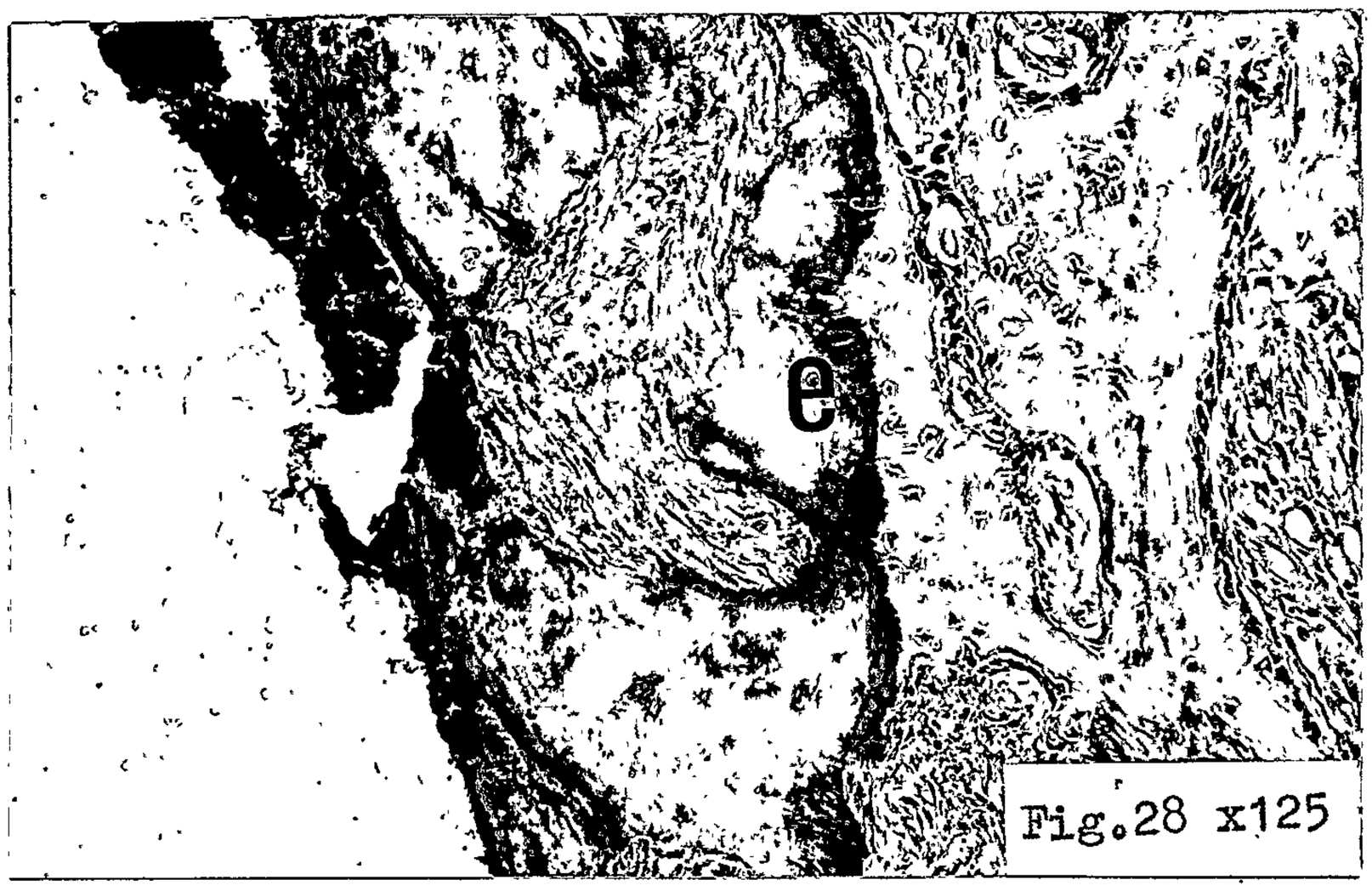


Fig.28 x125

Tooth treated	Treatment	Period of observation	Results
1. LR 4th Pm.	Immediate root filling of both roots to cemento-dentinal junction with gutta percha and root sealer.	6 months	Periapical tissues normal.
2. LL 3rd Pm.	Immediate root filling of mesial canal to cemento-dentinal junction with gutta percha and sealer; distal apex perforated and overfilled.	14 months	Mesial lamina dura intact. Encasement of extruded material distally with fibrous tissue.
3. LR 3rd Pm.	Immediate root filling of mesial canal to cemento-dentinal junction with gutta percha and sealer; distal perforation and extrusion of gutta percha.	12 months	Mesial periapex normal. No reaction to gutta percha distally.
4. LR 4th Pm.	Root sealer passed into periapical tissue through distal perforated apex.	2 weeks	Slight chronic inflammation around sealer.

Discussion

It is self evident that far reaching clinical inferences can not be made from the limited material presented in this section, but it is nevertheless of interest to compare the results with previous investigations reviewed above.

Immediate root filling to the cemento-dentinal junction in four dog root canals using gutta percha and root canal sealer resulted in healing of the apical tissues. Short term experiments were not pursued in an attempt to establish whether transient inflammation occurs in the apical ramifications or periapical tissues following pulpectomy, but after 6 months there was no evidence of cellular disturbance in the soft tissues apical to the root filling. In each case, vital tissue was retained in the apical ramifications. There was no indication that resorption or remodelling of apical cementum occurred following root filling, and stenosis of the ramifications by newly formed hard tissue was not observed. It therefore appears that the healing process in the dog following root filling to the dentino cemental junction is less complicated than the reparative process commonly observed in human teeth similarly treated.

Cementum in contact with the root filling material appeared normal, and this conflicts with the findings of Erasquin and co-workers[79] who ascribed cementum necrosis in root filled rats' teeth to the toxicity of the zinc oxide based sealer. Further investigations are required with the dog to ascertain whether cementum necrosis - reflected in the lack of staining of cementocyte nuclei - may occur following root canal filling of sterile canals. Following perforation of root apices in the dog, however, necrosis of cementum was observed together with extensive resorption, remodelling and anchylosis to bone. This may be a response to an alteration in functional requirements, although direct contact with the filling material and the presence of periapical inflammation could be additional factors influencing the remodelling of the cementum. The extrusion of a gutta percha point with a small

amount of root canal sealer into the periapical tissues was well tolerated and there was no inflammatory reaction present after 12 months. The tendency for a connective tissue capsule to form around gutta percha has been reported by Muruzabal et al[174] in a study of periapical overfilling of molar root canals in the rat, and a similar response in the dog is not unexpected. In this experiment, the gutta percha point compressed the inferior dental nerve (fig.25) but the pronounced atrophic changes which apparently occurred in inferior dental nerves in rats following similar traumatic impingements[174] were not observed in the dog.

Comparatively little inflammatory response was observed 2 weeks after overfilling a canal with a large quantity of zinc oxide based root sealer, and construction of bony trabeculae by osteoblasts in close proximity to the material (fig.29) suggests that it may not be as highly toxic as some investigators suggest.[78,220] In a similar experiment, after 14 months, the extruded material was found to be encased in dense fibrous tissue and inflammatory cells were virtually absent. Scattered macrophages which were loaded with particles indicated that slow resorption of the material was occurring, but there was no macroscopic diminution of the material observed on radiographs during the period of investigation.

These experiments suggest that overfilling with gutta percha or zinc oxide based root sealer does not invoke a sustained inflammatory response in the periapical tissues of the dog, and further investigations may confirm the findings in this study that definitive healing is by fibrous tissue encapsulation.

Experiments in which canals were intentionally underfilled to various levels were not pursued. The dog pulp is readily dislodged from the root canal and attempts at partial extirpation by hand instruments invariably resulted in total pulpectomy. However, the work of Ostby[185] concerning the role of the blood clot in underfilled canals could be extended using the dog as a subject. Following perforation of the root apex and haemorrhage into the canal, root filling to various levels could be undertaken with the object of studying the pattern of healing.

SECTION 2

The infected root canal

From the early days of endodontic therapy, researchers have emphasised the necessity for eliminating infection from root canals as a prerequisite for successful results. In an infected root, the organisms are situated for the most part in the main root canal and the numerous accessory canals[9,238] and as a result of their chemical activities, leucocytes are attracted to the area and surround the openings of the canals. In the canals the organisms are protected from attack by the leucocytes and the diffusion into the tissues of toxic bacterial metabolites frequently results in bone resorption and the appearance of a granuloma. Fish[85] describes three well defined zones of tissue reaction surrounding the infected apex - a contaminated zone, a zone of irritation and a zone of stimulation. This is the situation existing in chronic infection, but the possibility of acute exacerbation and invasion of the periapical tissues by organisms of enhanced virulence must be borne in mind.

Grahnen and Hansson[95] studied the results of 1277 root treatments and found that endodontic treatment of infected root canals has a higher failure frequency than treatment of roots with vital pulps. Seltzer and co-workers[221] assess the failure rate in teeth with apical areas as 2½ times greater than that following vital pulpectomy. The difficulty in obtaining apical healing is in many instances due to an inability to eradicate the bacteria, but it must be pointed out that the research work of some recent investigators has caused a reassessment of the compulsory condition of sterility as a prerequisite for success[159]. Hobson[121] reviews the bacteriological problems of root canal therapy and questions whether the elimination of bacteria is possible or essential. If not, what type of organism can be regarded as non pathogenic? A review of the literature reveals that a lack of knowledge surrounds the interrelationships between the pathologic condition, the clinical signs and symptoms and the infecting organisms.

Investigators have attempted to correlate the bacteriological condition of the canal at the time of filling and the subsequent

success rate, and some studies strongly favour the root filling of canals which have yielded a negative culture. Buchbinder[36] reported significantly better results when culturing was used, and Morse and Yates[168] stress the necessity of acquiring two negative cultures before root filling. Oliet[178] showed that a greater degree of success in healing occurred when teeth were filled with no evidence of micro-organisms in the canal. Appleton[4,5] concluded that a case of periapical infection which yields a negative culture before filling the canals has a more favourable chance of clinical and radiographic success than does a case whose canals are filled while the cultures are still positive. He found 94% success with teeth whose canals were filled after sterilisation as judged by negative cultures, while canals which were filled when some infection remained showed a drop in success rate to 85%. An important point however, is that considerable success can be achieved when some infection persists in the root canal.

Zeldow and Ingle[256] instance the greater success rate which occurs in teeth filled after obtaining a negative culture. Success was achieved in 83.3% of teeth while prefilling cultures revealed persisting infection, while 94.5% success was observed in teeth where prefilling cultures were negative. These results further demonstrate that the presence of bacteria does not preclude success.

Seltzer, Bender and Turkenkopf[219] found, in a review of 2921 root canal treatments, that in teeth without rarefaction there was no statistically significant difference between success of repair in teeth yielding positive cultures at the time of root filling and those yielding negative cultures. They conclude that since root canal therapy is as effective in teeth whose canals yielded positive cultures as in those whose cultures were negative, the drastic use of irritating drugs to rid the canal of the last trace of micro-organisms does not seem justified.

In a further communication[16], these investigators concluded that no advantages were shown to accrue from obtaining a negative culture and they no longer consider the culture technique as necessary. In an analysis of 2335 root canal therapy cases, the presence or absence of bacteria in the canal prior to filling

appeared to have surprisingly little effect on the eventual outcome. The degree of success appeared to depend more on the method and completeness of filling than on conditions of the culture.

Maisto[153] does not subscribe to bacteriologic culture procedures and claims equally good results without the use of c@lture techniques.

Seltzer et al[221] state that the culture has been found to be an unreliable indicator of root canal sterility. Hobson[121] also comments on the inadequacy of the present culture method. The paper point may not contact all the organisms in the canal and if too few bacteria are present in the inoculum, as may be the case immediately after instrumentation and irrigation, a false negative result may be obtained. The culture procedures currently employed probably seldom reveal the complete bacterial flora of the canal, for special growth requirements are required for many organisms and all bacteria will not grow in the same medium. The culture medium should be capable of supporting aerobic and anaerobic growth, and Shovelton[225] warns that if cultures are incubated aerobically only, anaerobes could persist in a canal which has yielded two negative results. Finally, the absence of visible turbidity in the culture tube does not mean that it is sterile. The medium may support bacterial growth and yet not appear turbid to the eye.

Several authors question whether it is possible to sterilise fully the main and lateral canals and tubules by present methods. The work of Stewart[236] and Auerbach[6] suggests that canal debridement and irrigation is an efficient means of clearing a large number of canals of infection, but Zeldow and Ingle[256] consider that any reduction in flora is transient. Coolidge[43] stated that complete sterilisation of the dentine is impossible by means of drugs, and permanent sterilisation of the root cannot be accomplished. The periapical response to experimentally infected and root treated teeth in dogs was studied by Matsumiya and Kitamura.[159] These authors infected dog root canals and then attempted to sterilise the canals by various means. Immediately

after attempts at sterilisation, bacteria could always be demonstrated histologically in apical ramifications, cemental lacunae and in tubules. They found that the sterilising influence of the various drugs employed was superficial and temporary. As the effect of the antiseptic decreased, deeply placed bacteria proliferated and spread causing reinfection of the canal. They conclude that attempts at sterilisation of a canal are fruitless, but they are of the opinion that bacteria in cemental lacunae and apical ramifications tend to die out in time following treatment and filling of the root canal, due to the bactericidal action of tissue fluids and the apical reaction.

It must be emphasised that infected cementum, which is found in the teeth of the dog, and not in the human, is probably impossible to sterilise by means of medicaments placed in the root canal, and the situation is more complicated in the dog as a result of this.

Kaneko[132] also used dogs and employed iontophoresis with various antibiotics in an effort to gain better infiltration of the drug with deep penetration and saturation of dentinal tubules and canal ramifications. He concluded that removal of bacteria in tubules and ramifications was impossible even using iontophoresis. He agrees with Matsumiya and Kitamura that bacteria do reduce in numbers during healing and he emphasises the importance of using a material in the root canal which induces rapid healing rather than expend effort trying to obtain perfect sterilisation.

Seltzer et al[220] elaborated on the investigations of Matsumiya and Kitamura. Using the experimentally infected teeth of dogs, they filled 24 canals which yielded prefilling negative cultures and 34 canals which supported bacterial growth. Comparison of healing indicated no discernible difference between the two groups. When repair occurred, it occurred whether or not organisms were present. When repair did not occur it was as likely not to occur around canals which were negative as around those which were positive. They found marked variability in healing. Some endodontically treated teeth were less successful than untreated controls.

This variability in the reaction of the periapical tissues in dogs' teeth following experimental infection of the root canals has been commented on by Snyder[232] as well as the above authors. He reported that results varied widely in the same animal, in the same quadrant and under the same conditions, and there did not appear to be a reliable pattern of healing following endodontic therapy.

In many instances the above mentioned investigators left the root canals open to the oral environment until areas of reaction developed and the variation in periapical response may have been partly due to differences in the flora which subsequently became established in the various canals. In the investigations described in the following pages, only the alpha haemolytic streptococcus was inoculated into canals in order to reduce the variation in periapical response. It became apparent however, that many factors influence the extent of the apical reaction, and variation in response to the same organism frequently occurred. Some infected canals were root-filled after areas of reaction had developed. Debridement of the canals was carried out but no attempt was made to sterilise the canals by drugs before root filling. The object of these experiments was to discern whether the reduction of the bacterial population by debridement was in itself sufficient to induce healing. These experiments were primarily designed to endorse the usefulness of the dog in the sphere of experimental endodontics where infected root canals are required, and in addition, to explore some aspects of the work of Matsumiya and Kitamura.

Experiment 5. Figs. 30 - 36 pages 45, 46.

Paper points, impregnated with a 24 hour culture of streptococcus viridans were inserted into the root canals of the lower left 3rd premolar (fig.30,e) and after three months, the bacteria had caused a periapical reaction with the appearance of radiolucent areas around both apices. (Fig.30). After sealing off the mesial canal as control, the distal canal was reamed and filed to the dentino cemental junction, irrigated with sterile water then root filled with gutta percha points and root canal sealer.

After 6 months, the periapical areas were still apparent (Fig. 31). There was a slight difference in Xray density and tube angulation between the radiographs depicted in figures 30 and 31 and an accurate assessment of the change in size of the apical areas was not possible. Comparison of the two radiographs suggested however, that the mesial area had progressed, whereas the distal area had regressed to a fraction of its original size. If this had in fact occurred, the root treatment, by eliminating masses of bacteria from the main root canal, created an environment conducive to healing.

A photomicrograph of the mesial root (Fig.32) showed the development of an extensive apical abscess with bone resorption and some liquefaction necrosis, and this reaction was considerably more vigorous than the inflammatory response surrounding the distal apex (fig.33). Indeed, active bone formation surrounded the distal inflammatory focus (inset fig.33). Cementum resorption had occurred in both apices. Gram stain showed that both roots were heavily infected with Gram positive bacterial forms in cemental lacunae (figs.34,35) but the cementum appeared more densely populated in the mesial root than the distal. Bacteria were also prevalent in the mesial root canal and apical ramifications. A high power photomicrograph shows Gram positive bacteria in the lacunae.(fig.36)

Experiment 6. Figs. 37 - 39, page 48

Inoculation of the canals of the lower left 3rd premolar with a 24 hour culture of streptococcus viridans had produced radiographically discernible areas of rarefaction around both root

Fig.30

Fig.31

Fig.32 x16

Fig.33 x16

Fig.34 x32
Gram. mesial

Fig.35 x32
Gram. distal

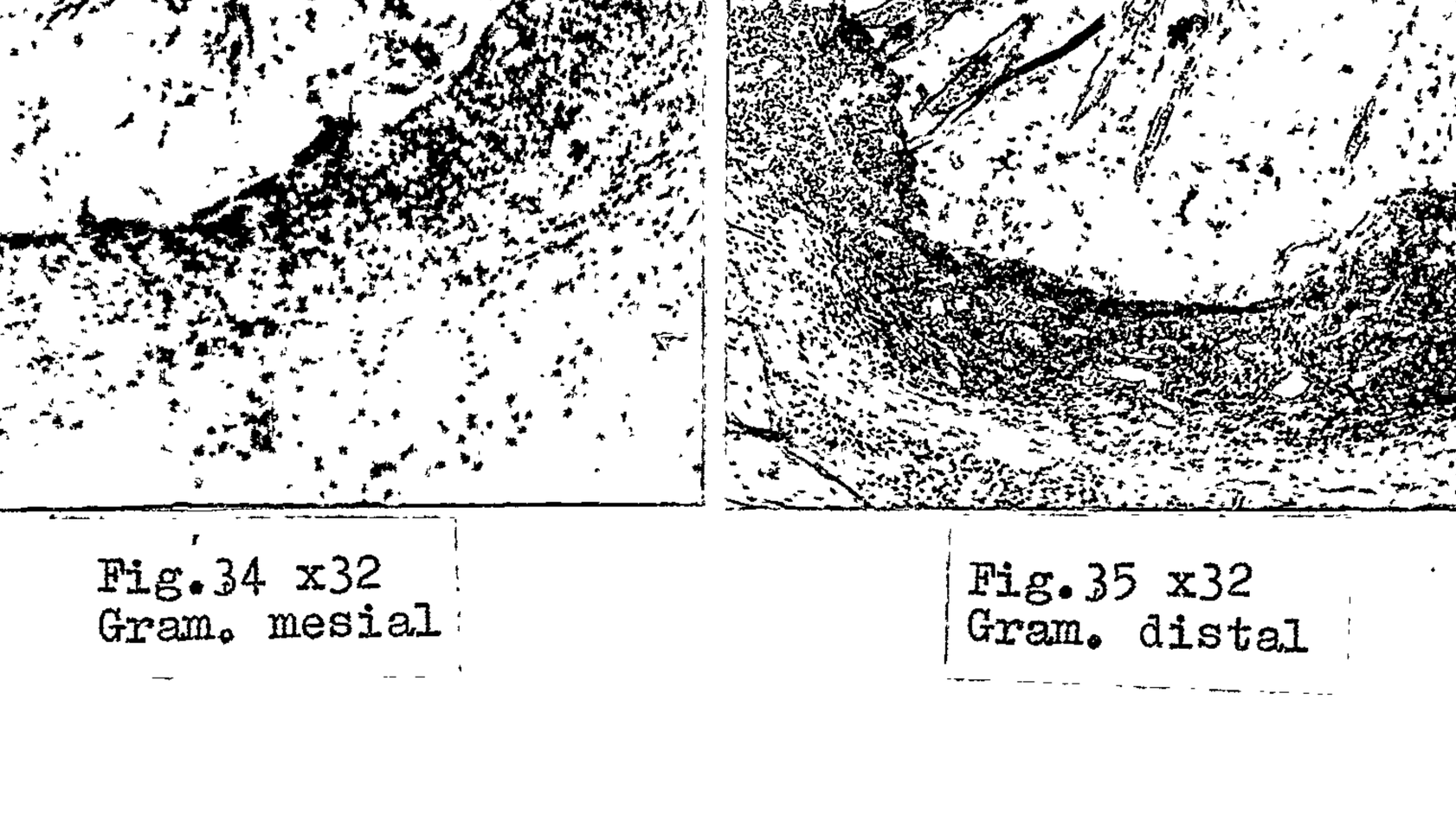


Fig.36 x560
GRAM

apices after 3 months (fig.37). The mesial canal was sealed off as control and the distal canal was reamed and filed to the cemento-dentinal junction. Root filling to this level was effected using root canal sealer and gutta percha points. Sterile water only was used to irrigate the canals during mechanical preparation.

The radiograph taken at the time of sacrifice 5 months later suggested that a slight reduction in size of the radiolucent area around the distal apex had occurred (fig.38) but the difficulty in assessing alteration in the size of an area of reaction from radiographs has already been referred to. Sections of the periapical region showed that the degree of inflammation around the distal apex was considerably less than that which had occurred mesially. The variation in apical response which follows inoculation of root canals with the streptococcus is frequently the explanation for such a difference, but radiographic and other considerations cited below suggested that in this case the root filling had resulted in a regression of the inflammatory reaction around the distal apex. There was evidence of extensive resorption of the distal apex and this might have been due to a pre-existing lesion of greater severity than seen at the time of sectioning. Radiographically, the apical area around the distal apex appeared far more extensive than was shown histologically. Serial sections indicated that small bony trabeculae scattered in the periapical region were probably recently formed, but were not of sufficient size or compactness to obliterate the radiolucent area from the radiograph. Bacteria were stained in the mesial apex but not distally.

These observations may signify that an inflammatory reaction of greater severity previously existed around the distal apex, and that root filling had permitted subsidence of the periapical disturbance to a level where bony repair could be initiated. Filling in with bone and healing on an X-ray picture would take a further period of time.

Fig.37

Fig.38

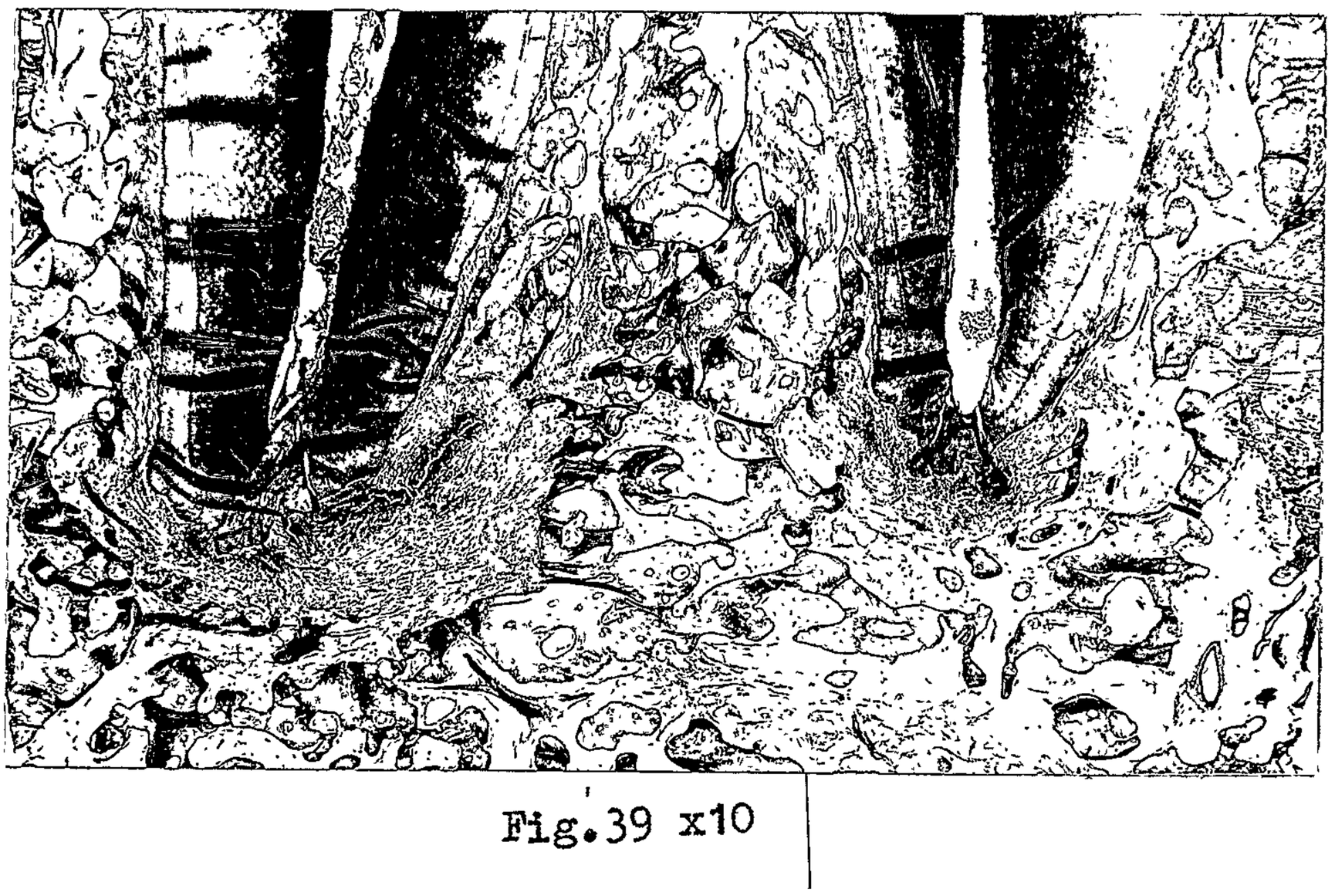


Fig.39 x10

D M

12

Fig.40

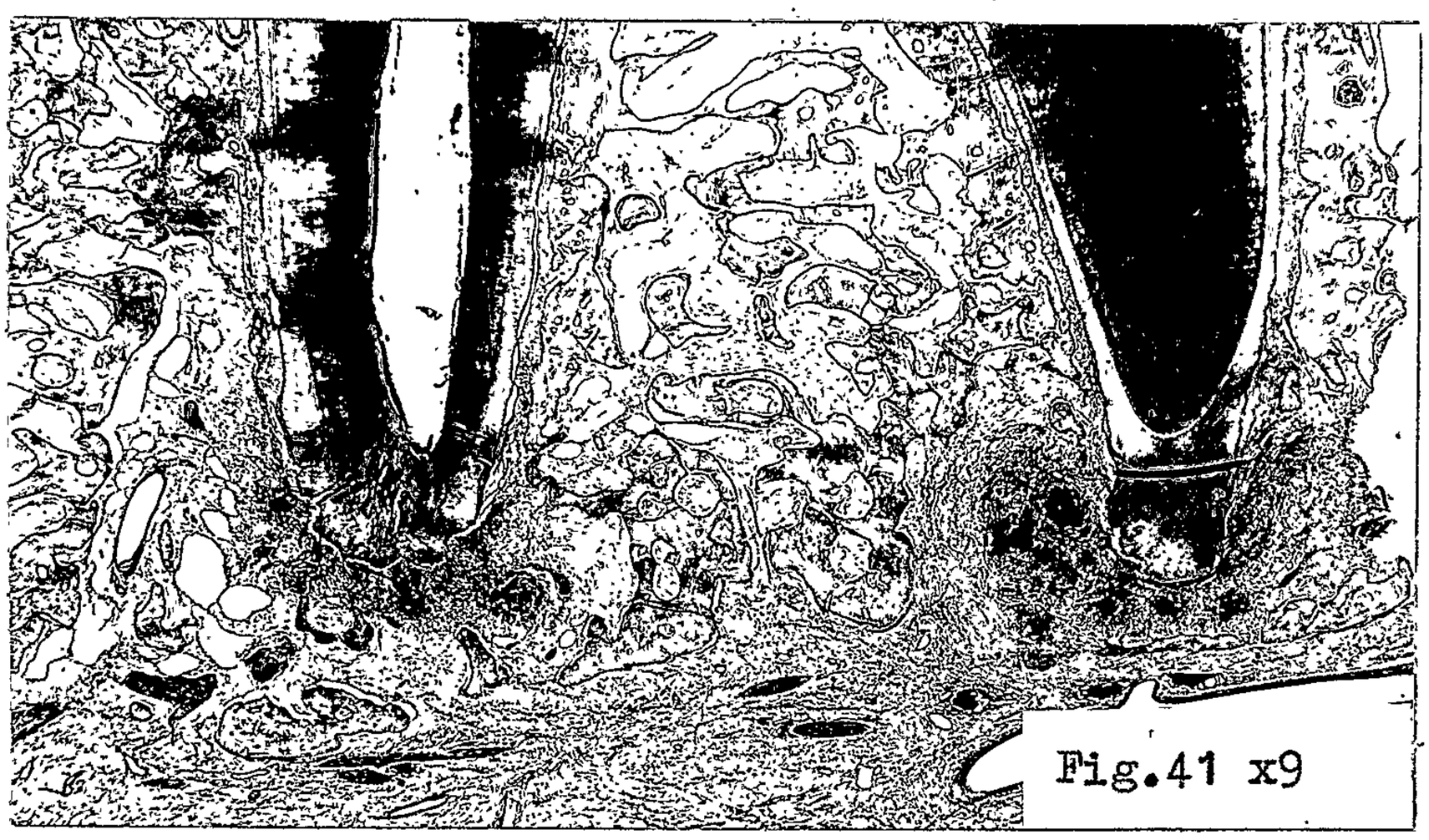


Fig.41 x9

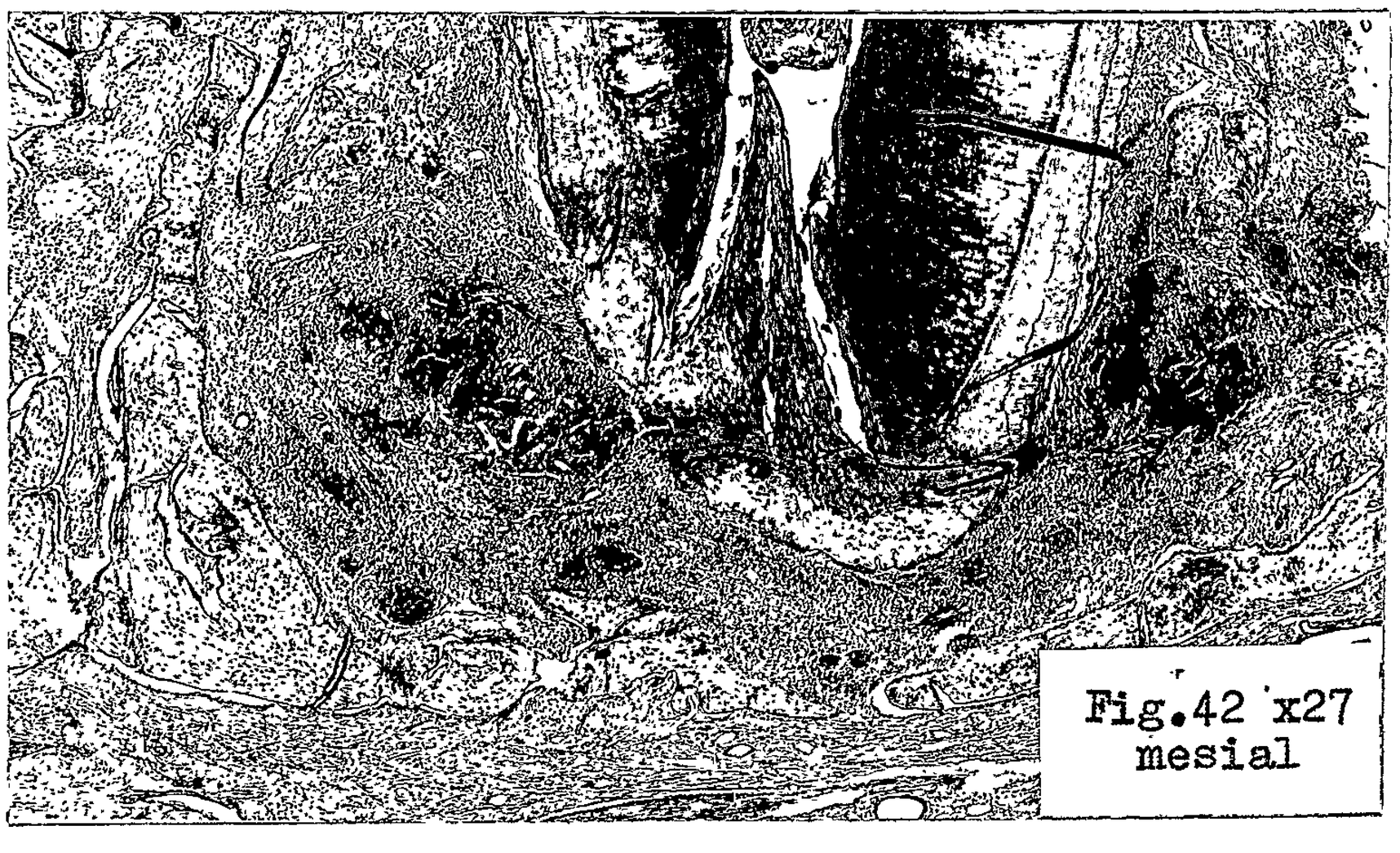


Fig.42 x27
mesial

Fig.43 x25
distal

Experiment 7. Figs. 40 - 43, page 49.

Following an incubation period of 2 months, streptococcus viridans had produced areas of reaction around the apices of the lower right 4th premolar. The mesial canal was sealed off as control and the distal apex perforated. Root filling was undertaken with gutta percha points and root canal sealer but no effort was made to extrude the filling material into the periapical tissues (fig.40).

After 2 months, sections showed that the reaction was still severe around both roots (figs. 41,42,43) although distally it appeared to be less extensive. Apical to the root filling material the reaction was slight (fig.43) but it was marked around lateral ramifications. Gram staining revealed heavy colonisation of these apical canals with Gram positive bacterial forms.

Experiment 8. Figs. 44 - 45, page 51.

A 24 hour culture of streptococcus viridans had been inoculated into both root canals of the lower right 3rd premolar and left for a period of three months. Large areas of reaction developed around the apices during this period (fig.44). The mesial canal was sealed off with amalgam as control and the distal canal (which yielded a positive culture) was enlarged by reaming and filing prior to root filling to the dentine cemental junction with root canal sealer and gutta percha points. This experiment was identical to Nos. 5, 6.

After 6 months, histologic sections showed an area of reaction around the mesial root apex which was greater in extent in deeper sections than the one shown (fig.45). There was complete healing around the distal apex and bacteria could not be detected. This is a case in which the elimination of gross infection from the main canal by debridement and root filling must have been followed by eradication of the remaining bacteria from lateral canals and cemental lacunae by defence mechanisms in the periapical tissues.

Experiment 9. Figs. 46 - 48, page 52.

Areas of chronic inflammation had been produced around both roots of the lower left 2nd premolar by streptococcus viridans after

Fig.44

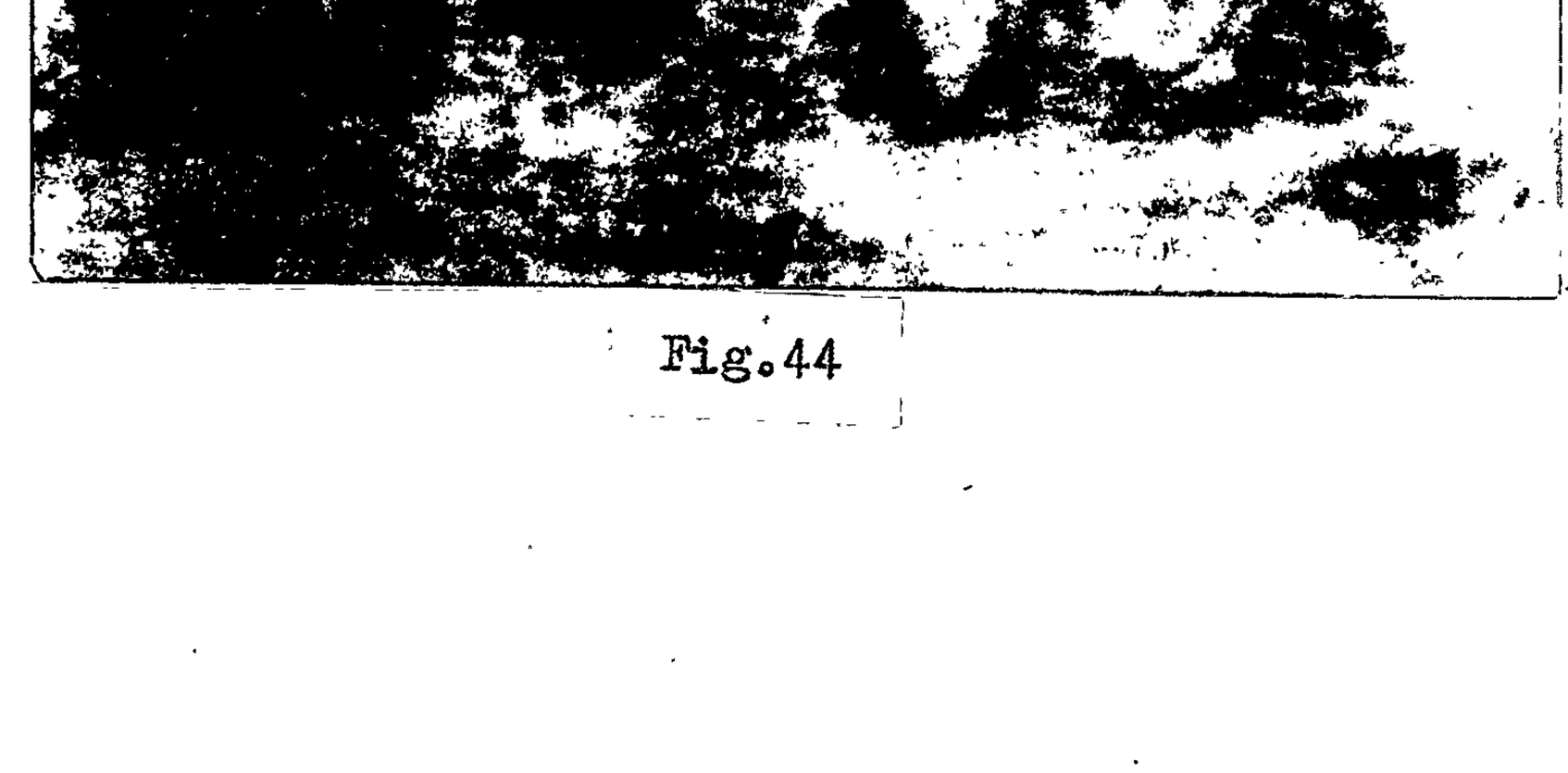


Fig.45 x12

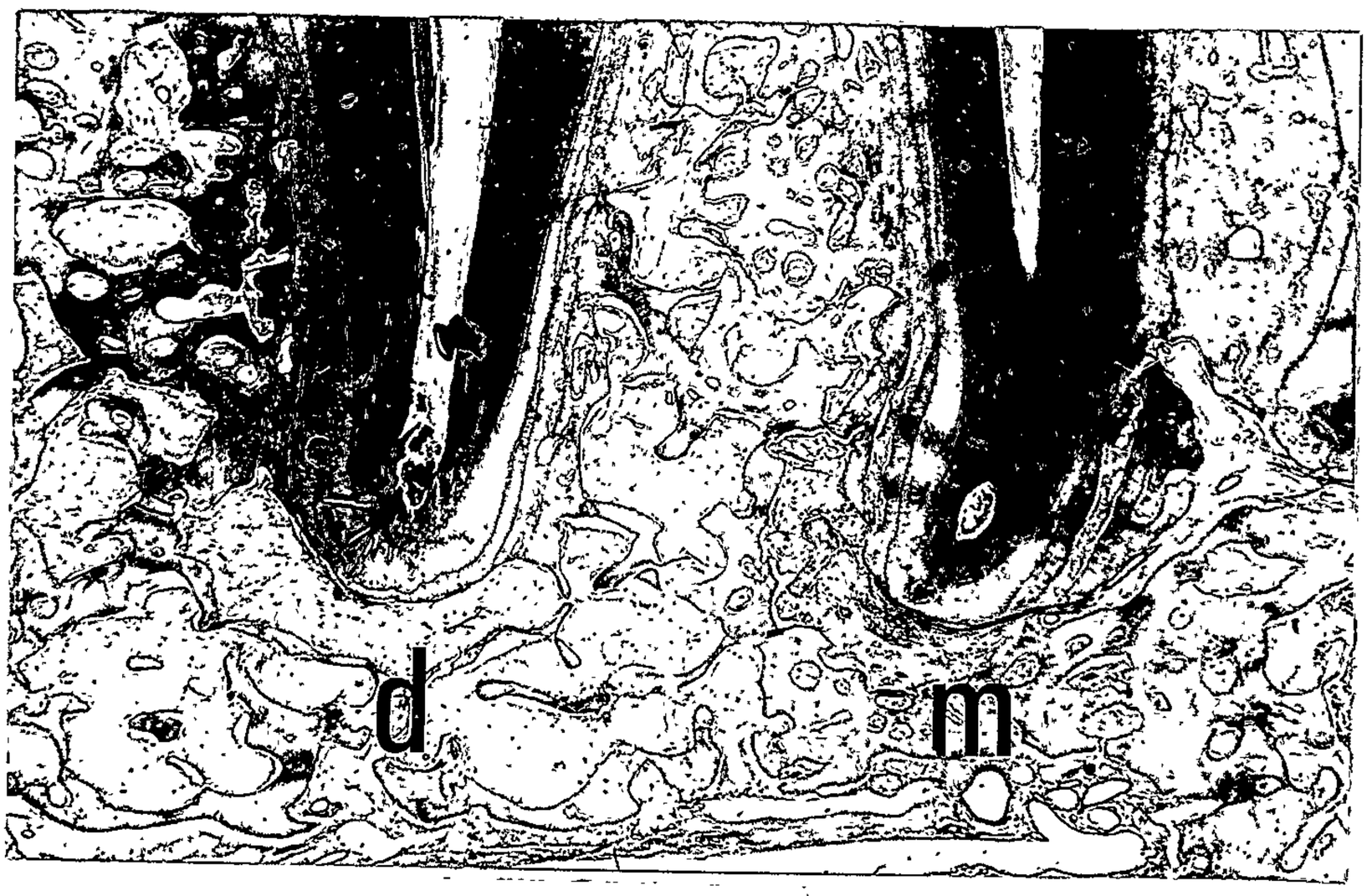


Fig.46 x9

Fig.47 x32
distal

Fig.48 x32
mesial

3 months. The mesial canal was sealed off as control while the distal canal was filled to the commencement of the apical ramifications following debridement. Immediately following root filling and for two days thereafter, 500,000 units of procaine penicillin was administered intramuscularly.

Following sacrifice 4 months later, the distal apex was seen to be free of inflammation (figs. 46,47) while the mesial periapex contained isolated foci of round cells (fig.48). This could have been due to the empty canal ramifications causing a hollow tube effect.[64] Bacteria could not be stained in either apex, and it is possible that the penicillin contributed to this reduction.

TABLE OF EXPERIMENTS

Tooth treated	Treatment	Period of observation	Results
5. LL 3rd Pm with apical areas of reaction due to inoculated streptococci.	Mesial control. Distal canal enlarged to cemento-dentinal junction and root filled with sealer and GP.	6 months	Mesial area probably enlarged; distal area possibly reduced in size. Bacteria stained in each apex.
6. LL 3rd Pm with apical areas as above.	Mesial control. Distal canal enlarged to cemento-dentinal junction and root filled with sealer and GP.	5 months	Partial subsidence of the distal reaction appeared to have occurred with evidence of bony repair. Bacteria not stained distally but mesial cementum infected.
7. LR 4th Pm with apical areas as above.	Mesial control. Distal apex perforated and canal root filled to apex with sealer and GP.	2 months	Reaction remained severe around both apices. Bacteria stained in each apex

8.	LR 3rd Pm with apical areas.	Mesial control. Distal canal enlarged and root filled to cemento-dentinal junction with sealer and GP.	6 months	Inflammation persisted around mesial apex. Distally, healing was complete. Bacteria stained only in mesial apex.
9.	LL 2nd Pm with apical areas.	Mesial control. Distal canal enlarged and root filled to cemento-dentinal junction with sealer and GP. Penicillin administered for three days.	4 months	Complete subsidence of inflammation distally. Bacteria could not be stained in either apex.

Discussion.

In this series of experiments where immediate root filling of infected canals was undertaken, no attempt was made to sterilise the canals with antiseptics. The bacteria resident in the main canal were reduced in number by reaming, filing and irrigation with sterile water, but bacteria in protected sites such as the apical ramifications and cemental lacunae were allowed to remain. These experiments were an addition to the work of Matsumiya and Kitamura[159] who observed that periapical repair of endodontically treated teeth in dogs was the same whether or not micro-organisms were present in the root apex. In the investigations herein described, canal debridement and irrigation were employed with the object of seeing whether these measures alone would reduce the bacterial population to a level at which the periapical tissues might be able to effect healing.

Of the five investigations reported, experiment 5, 6 and 8 were similar in that root filling of canals, with apical areas of reaction, was effected to the cemento-dentinal junction with root canal sealer and gutta percha points. Histologic examination of the periapical tissues around the control and treated roots was carried out after 5-6 months. In one instance (expt. 8) complete healing occurred and bacteria could not be detected in the treated apex. In both the other experiments, it was considered likely that some reduction in the extent of the inflammatory reaction around the treated apices had occurred concomitant with a decrease in the bacterial population. These results are not in conflict with the findings of Matsumiya and Kitamura. The eradication of bacteria from apical ramifications and cemental lacunae by placing medicaments in the main root canal may well be impossible. Provided the main root canal is thoroughly debrided and then hermetically sealed to prevent any reinfection, it is possible that the number of bacteria remaining in protected sites would tend to be reduced by the action of the inflammatory exudate and this would permit healing to occur in a proportion of cases.

The possibility that the periapical area of reaction with

its inflammatory exudate can effect sterilisation of cemental lacunae and apical ramifications has been discussed by several authors. Blayney[23] states that the formation of a mass of granulation tissue about the root end should be considered a defensive effort on the part of the body. Within this small region, forces have been concentrated to resist further injury to the tissues, and after root filling, the defence cells in this area have the potential for effecting repair. There is no need to sterilise this granulomatous mass. Boyle and Harndt[34], Shindell[224], Kronfeld[140], and Grossman[96,97], determined that granulomata are sterile in a large percentage of cases, although the findings of Hedman[115] are at variance with the other authors.

Cahn[38] discusses the constituents of the inflammatory exudate and states that the inflammatory reaction is probably the best antiseptic substance we can command. In the Carrel Dakin method of wound treatment, wound closure is considered safe if bacterial activity is depressed to the point where the defence of the tissues can cope with the residual infection. Absolute sterility is not the sine qua non. In a tooth with necrotic pulp tissue, the source of bacterial growth is removed by canal enlargement and debridement and the tooth is rested as soon as possibly by filling. The cells of the apical granuloma will then perform their defensive duties. Frequent canal dressings, considers this author, open the parts to contamination and prolongs healing. Cahn believes that culturing the canal is unnecessary to determine the time of root filling except where there is persistent discharge and the causal organism must be isolated.

It appears then, that residual bacteria in root apices remaining after root filling do not necessarily militate against repair, and in a proportion of cases die out probably as a result of the action of the inflammatory exudate. However, the variability of bacterial virulence and the resistance of the host are factors which prevent generalisations concerning the fate of all residual bacteria.

When filling a canal which has harboured a putrescent pulp, Cahn advocates the use of systemic antibiotics. In one experiment reported above (expt. 9) parenteral administration of procaine penicillin followed root filling of an infected canal. After 4 months virtually complete healing had occurred around both treated and control apices and bacteria could not be detected by Gram staining. Provided the bacteria are sensitive to the antibiotic used, it is possible that systemic administration of these agents will enhance the action of the inflammatory exudate against bacteria in sites to which tissue fluids can diffuse. Further experimentation is required to elucidate this contentious issue.

In experiment 7, the treated root apex was perforated prior to root filling and after 2 months, the inflammatory reaction was still severe, although less in extent than seen around the control apex. It is probable that the injection of infected tooth particles into the periapical tissues during perforation with the Beutelrok drill was in part responsible for the continued severity of the reaction.

The observation period in these experiments was relatively short following root filling, but is a matter of speculation as to whether a more prolonged postoperative interval would have allowed resolution to occur around more of the treated teeth. Dixon and Rickert[66] who treated infected dog root canals with formocresol and root-filled them following two negative cultures, found residual inflammation persisting in some cases after 1½ years. They consider that longer periods of time are needed for complete resolution. Curzon[56] states that bone repair frequently is apparent in human cases after 6 months and healing is often complete within 2 years. In human teeth, Strindberg[239] found that repair of lesions following root canal therapy usually occurs after 6 months to one year but concluded that a 4 year observation period was the earliest on which results could be based. Bender et al[17] found that complete restoration of apical bone occurred only in 39.2% of 365 root canal treatments complicated with areas of apical rarefaction. Grossman[99] found complete elimination of areas in

62% of cases. In many instances there appeared to be a reduction in size of the area in 6 months but a residual radiolucency persisted and there appeared to be no significant alteration in extent of repair between 6 months and 2 years. Bender et al[17] discuss the significance of a persisting small focus of chronic inflammation apically and minimise its importance. They consider that the presence of lymphocytes and plasma cells apically may indicate a local tissue antigen/antibody response and instance the prolonged, symptomless presence of such cells in many bodily tissues, including the pulps of restored teeth.

There is no way of ascertaining, however, whether bacteria continuing to reside in protected sites in root apices are responsible for the continued reaction, a reaction which may, after years of clinical quiescence, undergo an acute exacerbation.

As to whether or not the foregoing observations in the human can be equated with the dog, Kukidome[141] stated that there is no basic difference between the healing of periapical tissues in the human and dog following treatment of infected canals.

SECTION 3

Resorbable Paste.

Since Walkhoff introduced resorbable paste (KRI 1) in 1928, the material has enjoyed a deal of populatity in the practice of endodontics. It disappears rapidly from the tissues and seems to excite a vigorous connective tissue response aiding in the rapid replacement of granulomatous tissue and the healing of apical areas. Since it also disappears from the apical portion of the root canal, and does not harden, it is usual to combine the filling of the apical area with the paste together with the obturation of the root canal by means of a hardening cement.

In spite of its widespread use there is a paucity of articles in the literature concerning the tissue response to this material when used in root canals, and a complete lack of information to support its occasional use as a pulp capping material.

Castagnola and Orlay in 1952[41] state that 'treatment with Walkhoff paste ..... is a reliable method resulting in a high percentage of success even in the most desperate cases. In fact, with sufficient patience on the part of the dentist and the patient, practically all teeth can be saved.'

Walkhoff paste is an iodoform paste which includes a mixture of parachlorophenol, camphor and menthol. Only minimal amounts of parachlorphenol are released from combination and Castagnola and Orlay state that its coagulating power may be disregarded, although its bactericidal powers - which they demonstrate to be wide - remain effective.

They list various favourable attributes of the paste. It does not irritate the periapical tissues and surplus pressed beyond the apex is readily resorbed. It is radiopaque, has a stable disinfecting power, and is readily inserted into and removed from the root canal. It also 'assists nature' to seal the canals with calcific tissue or with connective tissue. In two cases, paste resorbed from the apical third of the canal is shown histologically to be replaced with connective tissue. Cementum is seen lining the

apical part of the canal. The authors definitely advocate overfilling in the case of granulomas and consider that the paste is not washed out or dissolved but is resorbed by the invading connective tissue. In an analysis of 1000 cases treated in the student's clinic, 63.9% success was gained in cases with a circumscribed apical focus and 72.7% success in cases without an apical area.

Two cases reported by Laws[147] support the claim of Castagnola and Orlay that the removal of paste from the apical portion of the canal is followed by replacement with vital tissue and the laying down of calcified matrix along the canal wall. Laws states that this calcified tissue on the canal walls is calcified repair tissue and not secondary cementum.

Extension of periodontal tissue into root canals following root filling with resorbable paste has been reported by other investigators (Matsumiya & Kitamura 1960[159]; Kuroiwa 1960[142]; Hyakusoki 1959[124]; Kukidome 1957[141]; Engel 1950[75]), but Ostby[185] considers that the extent of this tissue ingrowth is limited.

Juge[130] in 1959 agrees that these pastes are particularly recommended in cases where the material used in filling is likely to penetrate beyond the apex, especially when the apex is wide open. He states that there is no clinical reaction to paste forced beyond the apex.

Juge[130], Laws[146] and Curson[54] recommend that the apex and periapex should be filled with KRI paste, but the bulk of the canal should be filled with a hardening paste and a gutta percha point. Hampson[105] recommends forcing the material not only into the periapical area, but through a sinus if present.

Wienreb[251] in 1960 recommends the use of another resorbable paste called Apicoflux containing iodoform, which after two negative cultures from the root canal, is inserted into the periapical area during several sittings until complete filling of the granuloma is achieved. When filled, the root canal is sealed with a non resorbable cement. He considers the denuded apex is a foreign body and surrounding it with a 'wound salve' is sufficient to initiate the

process of healing. The direct treatment of periapically involved areas with a resorbable paste seems to be helpful, claims the author, in bringing these areas more speedily to resolution.

Curson[54,55], speculates on the rapid disappearance of KRI paste from the tissues. He considers that the material disappears so rapidly that it probably leaches away in tissue fluids with only limited removal by the slower process of phagocytosis. Iodoform pastes, he concluded, are not suitable for filling a canal because they soon leave a void. Their insertion into periapical areas appears justified however, because the material seems to induce a reaction that leads to its replacement by connective tissue and speeds the repair of the abscess cavity.

With regard to the removal of the paste from the tissues, Laws[147] refers to the work of Kirk who described phagocytes with changed cytoplasm about implants of the paste in connective tissue and muscle of the rat. Ostby[185], however, states that the resorbable material is removed either by being dissolved in the tissue fluids or by being broken down and digested by free cells.

Muruzabal et al[174] studied the outcome of overfilling molar root canals in the rat. Extruded Iodoform paste was first surrounded by polymorphs then a few days later macrophages simultaneously removed leucocytes and paste. They state that the paste disintegrates in the periapex provoking a more intense tissue reaction and faster resorption.

Investigation is therefore required into the mechanism of paste removal from the tissues, the extent to which connective tissue ingrowth occurs following the removal of the paste from root canals, and the ability of the material to stimulate repair following the overfilling of infected root canals. A framework of experimental methods was devised to assess the suitability of the dog for this aspect of endodontic research.

Experiment 10. Figs. 49 - 52, page 64.

Insertion of streptococcus viridans for 3 months into the mesial and distal canals of the lower right third premolar (e) resulted in small areas of rarefaction around each root apex. The root apices were perforated and KRI paste was carried into the periapical tissues by means of a Lentulo spiral instrument (fig.49). Gutta percha points were inserted into the canals short of the apex, and immediately following root filling, 500,000 units of procaine penicillin were administered intramuscularly. This therapy was continued on the succeeding two days.

Four months later, the paste was seen to be resorbed well up the distal canal and to a lesser extent up the mesial root to a level corresponding to the point of termination of the gutta percha (fig.50). Histological examination (fig.51) showed connective tissue extending into each canal. There was some resorption of the cementum on the mesial aspect of the distal root, but this was not an uncommon event when apices had been perforated as well as surrounded by an inflammatory reaction.

A focus of residual paste persisted deep to the apex of the distal root (fig. 51,p). High power examination revealed intra-cellular particulate matter indicating phagocytosis of the remaining paste (fig.52). Gram positive bacteria could not be detected in the root apices.

Periapical healing in this experiment was complete but it was impossible to delineate to what extent the administration of penicillin, the natural defence machanisms of the periapical tissues and the iodoform paste assisted in achieving this result.

Experiment 11. Figs. 53 - 57, page 65.

Periapical granulomata were produced around the mesial and distal apices of the lower right fourth premolar (fig.53) following the inoculation of the canals with streptococcus viridans 5 months previously.

The mesial root canal was enlarged by reaming and filing to the cemento-dentinal junction and irrigated with sterile water. No

Fig.49

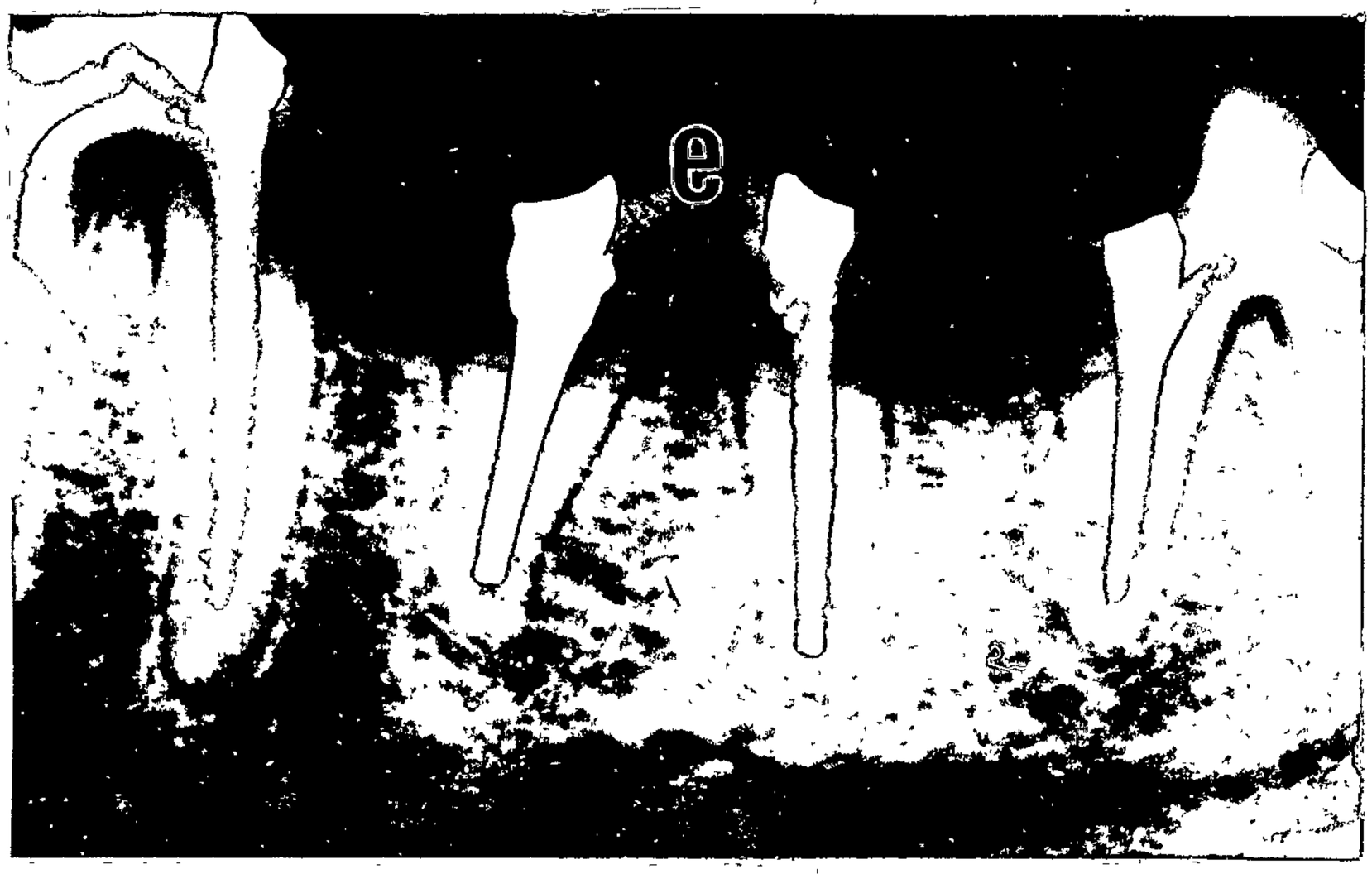


Fig.50

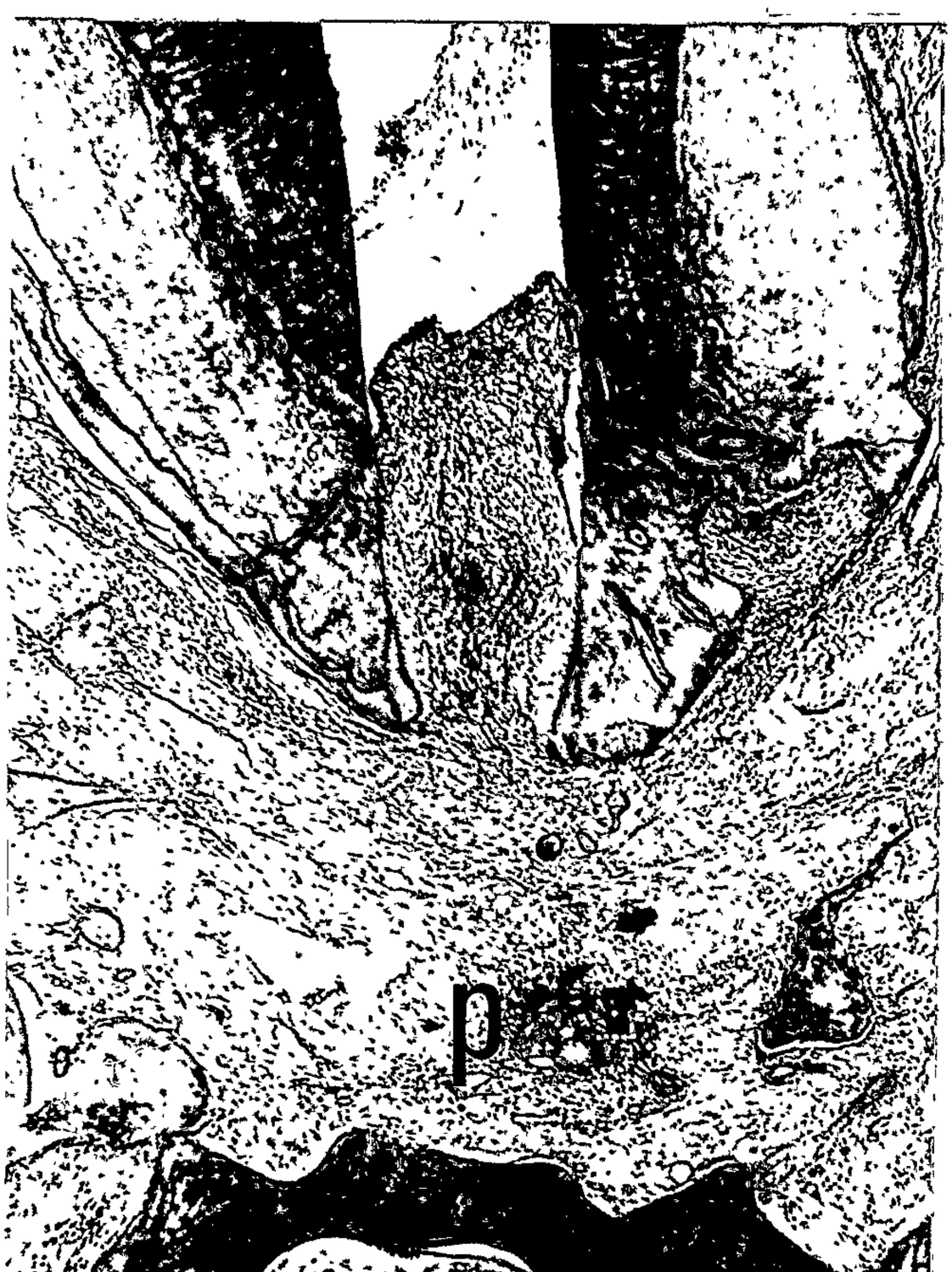


Fig.51 x32

Fig.52 x560

Fig.53

Fig.54

D M.

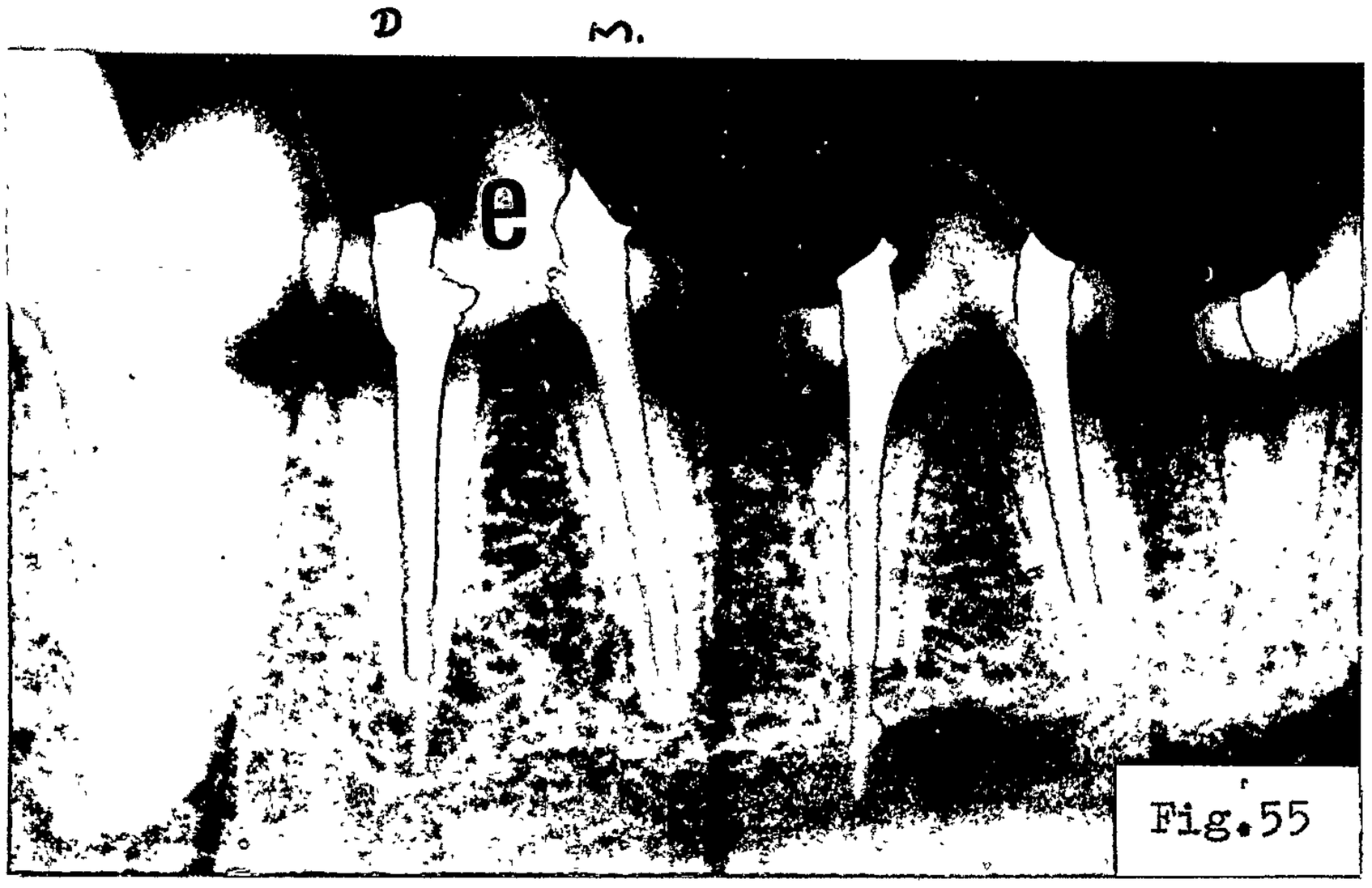


Fig.55

Fig.56 x32
distal

Fig.57 x32
mesial

attempt was made to sterilise the canal apart from mechanical debridement. Root filling was carried out with root sealer and gutta percha points.

Distally the apex was perforated with a Beutelrok drill and KRI paste was forced into the apical area. A gutta percha point was inserted past the apical foramen (fig.54).

After 7 months, the areas appeared to be healed on the radiograph (fig.55,a) and this was verified on histological examination. Mesially there was complete healing and the periapical bone had been restored to normal architecture (fig.57). Distally there was marked fibrosis around the extruded point and some epithelial proliferation, but new bone had filled in the pre-existing area (fig.56). It will be noted on the radiograph that the paste had been removed from the apical portion of the canal leaving the gutta percha point denuded. The mechanism by which the paste was removed could not be determined from these sections.

Bacteria could not be stained in either apex and it is probable that the antibacterial constituents of the inflammatory exudate eradicated the organisms from the apical ramifications and cemental lacunae and permitted healing to occur. Healing was complete around both apices, and resorbable paste in this experiment offered no advantages over root sealer and gutta percha.

Experiment 12. Figs. 58 - 65, page 67, 68.

In this experiment both apices of the lower right 4th premolar were perforated. Through the mesial apex, KRI paste was extruded together with a gutta percha point, while distally, root canal sealer was forced into the apical tissues (fig.58). The report on the distal perforation is found on page 34.

The animal was sacrificed after 2 weeks and the radiograph taken at that time showed that approximately 70% of the KRI paste had been resorbed (fig.59). The mechanism of this extremely rapid clearance of the paste from the tissues was not clearly defined. Sections of the submandibular lymph glands associated with drainage from this area showed heavy deposition of dark substance throughout the stroma (figs. 62,63), but it is unlikely that these deposits

Fig.58

Fig.59

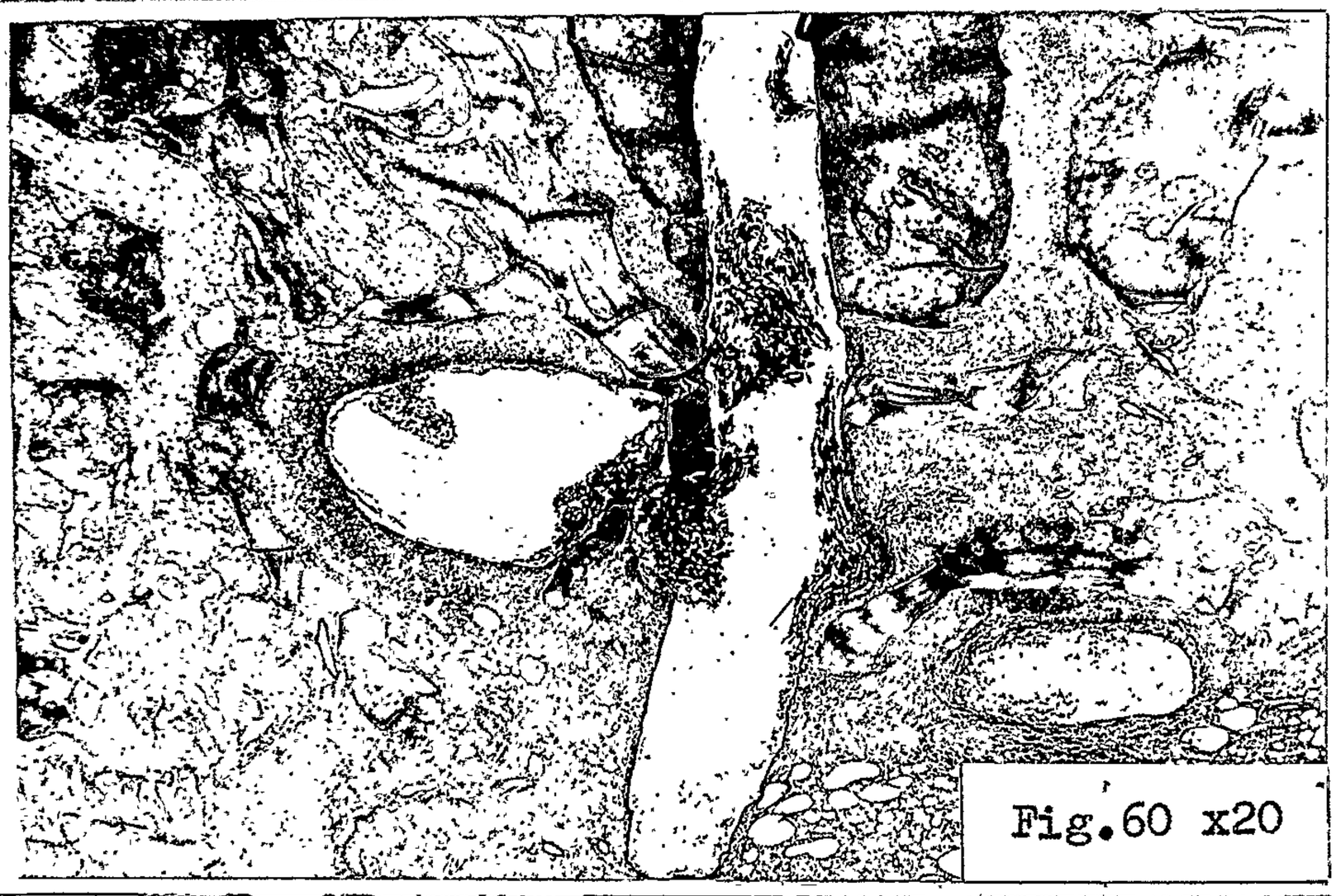


Fig.60 x20

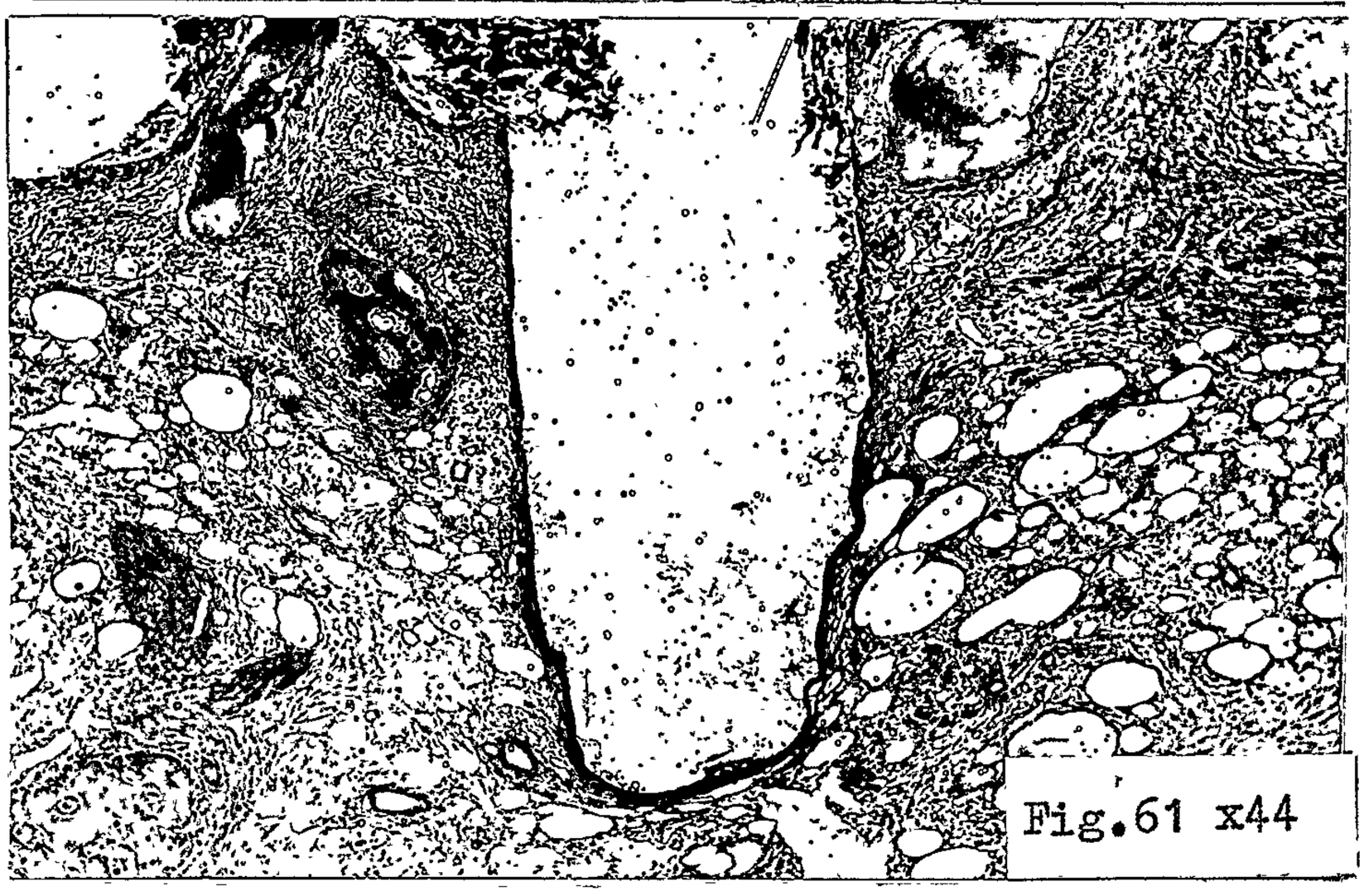


Fig.61 x44

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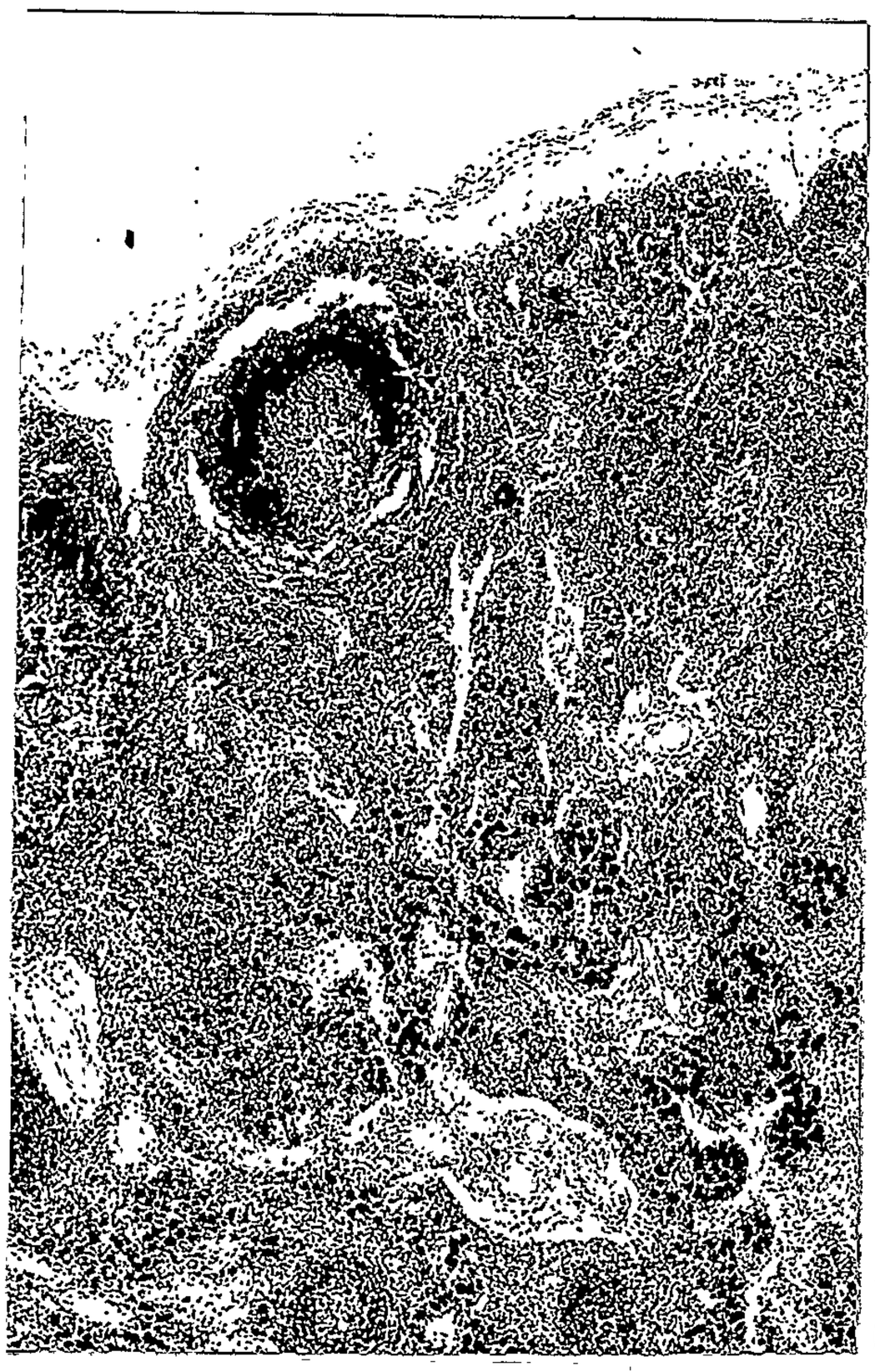

Fig.62 x32

Fig.63 x560

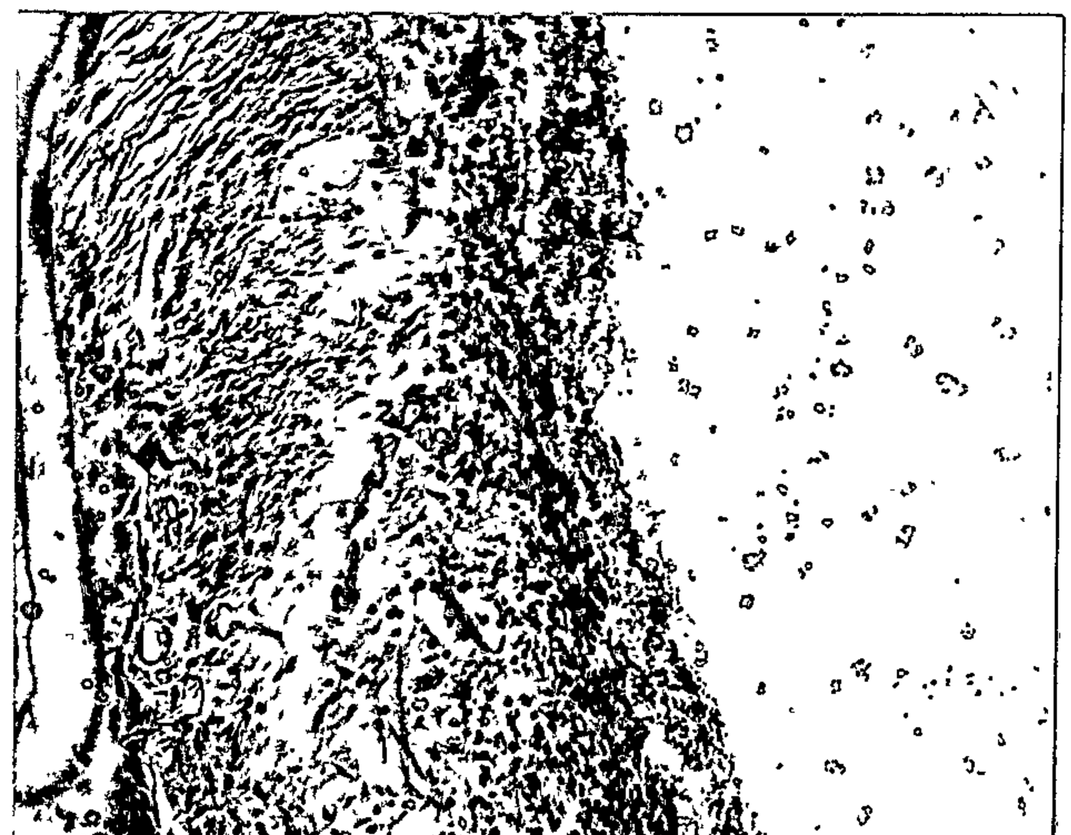

Fig.64 x125

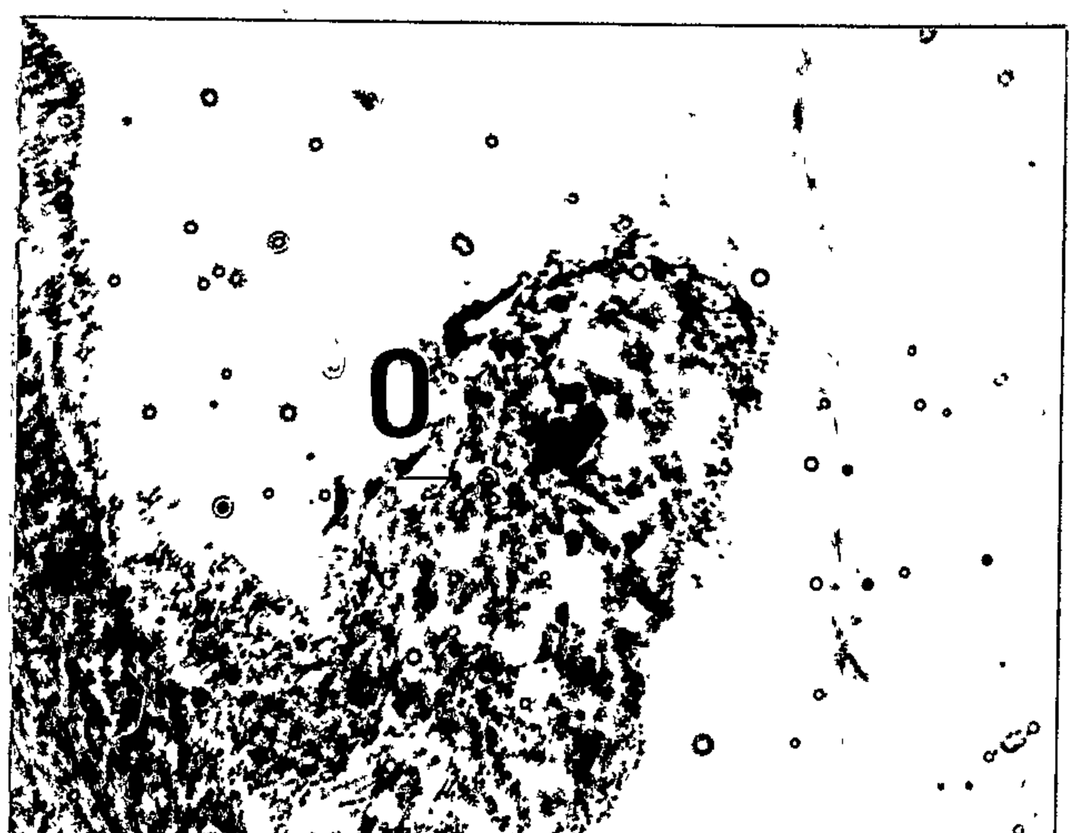

Fig.65 x125

represented material in macrophages which had travelled from the periapical tissues. The cells in the lymph gland were large and completely filled with dark staining material and similar cells were virtually absent from the periapical tissues surrounding the paste, although there was an extensive and dense aggregation of small cells around the paste. Since phagocytosis was not a dominant feature, and the paste was undergoing remarkably rapid clearance, it seems likely that much of the material may have drained directly by way of the lymph stream to the gland where ingestion by the endothelial cells lining the sinuses occurred.

Histologic processing dissolves free KRI paste from the tissues and most of the gutta percha had been dislodged during sectioning (figs. 60,61). Throughout the sections of the periapical tissues however there was occasionally seen some black staining substance, similar to that observed in the lymph gland, but small in amount, and it is possible that this material was intracellular (figs. 64,65). In one section (p) (fig.64) two large aggregations of this substance were seen and it is possible that they were loaded macrophages. In another area, (o) (fig.65) small darkly staining particles appeared to be retained in small cells. It will be noted that the cells at the periphery of the paste were small and closely packed and bore little resemblance to macrophages.

This experiment, while not fully elucidating the method by which paste was removed from the tissues, suggested that two mechanisms operated concurrently, viz., direct drainage to lymph glands following dissolution in tissue fluids, and a small amount of phagocytosis by cells of varying size.

This experiment further illustrated how well a denuded gutta percha point is tolerated by the tissues. In this case a sheath of fibrous tissue surrounded the point and areas of osteogenic activity were discerned in close proximity (fig.61).

Experiment 13. Figs. 66 - 71, page 70, 71.

Following pulpectomy and perforation of each of the root apices of the lower left third premolar, the root canals were flooded

Fig.66

Fig.67 x9

Fig.68 x32

Fig.69 x125

Fig.70 x32

Fig.71 x125

for 10 minutes with nutrient broth containing a 24 hour culture of streptococcus viridans. The mesial canal was then immediately overfilled with root canal sealer and a gutta percha point and the distal canal received a filling of KRI paste with a short gutta percha point. The radiograph taken at the time of sacrifice 6 months later (fig.66) showed that the KRI paste had been resorbed up the distal root canal.

Healing in both instances was observed in spite of the heavy bacterial contamination prior to root filling. Bacteria could not be stained in either root apex.

Mesially the extruded paste was well tolerated by the apical tissues, mainly being encased in fibrous tissue with very little inflammatory response (figs. 67,68). Bone trabeculae were seen in close proximity to the paste. A high power photomicrograph (fig.69) showed fibrous tissue interposed between the root sealing paste and the side of the root canal.

Distally there had been resorption of the KRI paste up the canal and replacement by healthy fibrous tissue. An unusual occurrence, which was not apparent in other instances of healing following the use of KRI paste in dogs teeth, was the layer of cementum which lined the apical part of the root canal (figs. 70,71). It will be observed on the low-power photomicrograph (fig.67) that dense fibrous tissue had replaced bone around the distal apex and this was noted on the radiograph as a slight radiolucency. In a clinical case, this could give rise to doubts as to the progress of healing in this area.

This experiment was partly designed to give some idea as to whether transient contamination during root canal procedures permit bacteria to establish themselves and subsequently cause periapical pathosis. A great deal cannot be deduced from the fact that the relatively innocuous streptococcus viridans failed to establish itself in the debrided canal in this experiment, except that an organism must be of high virulence to find a nidus for growth on introduction into the debrided canal during operative procedures.

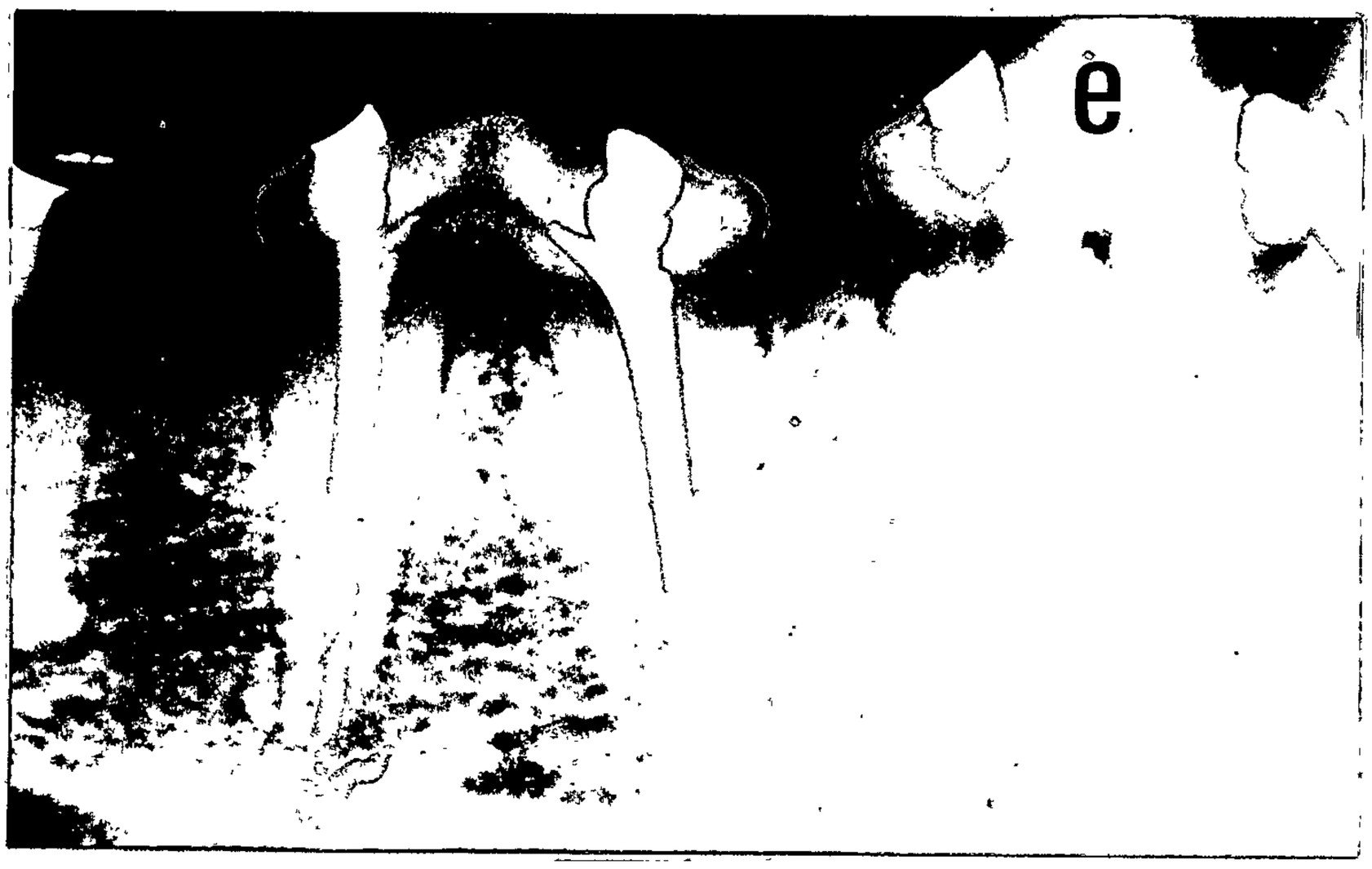

Fig.72

Fig.73

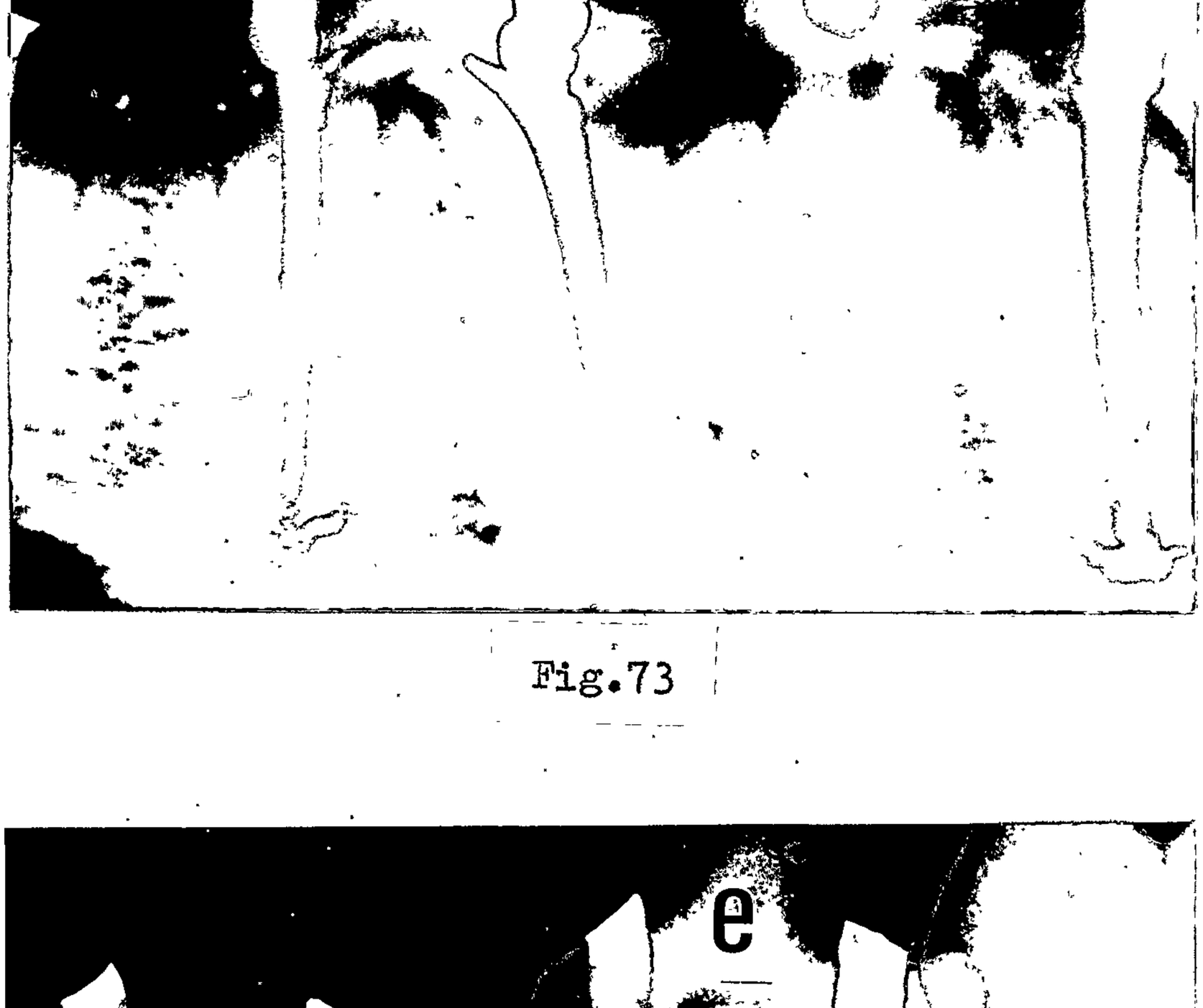

Fig.74

Fig.75 x9

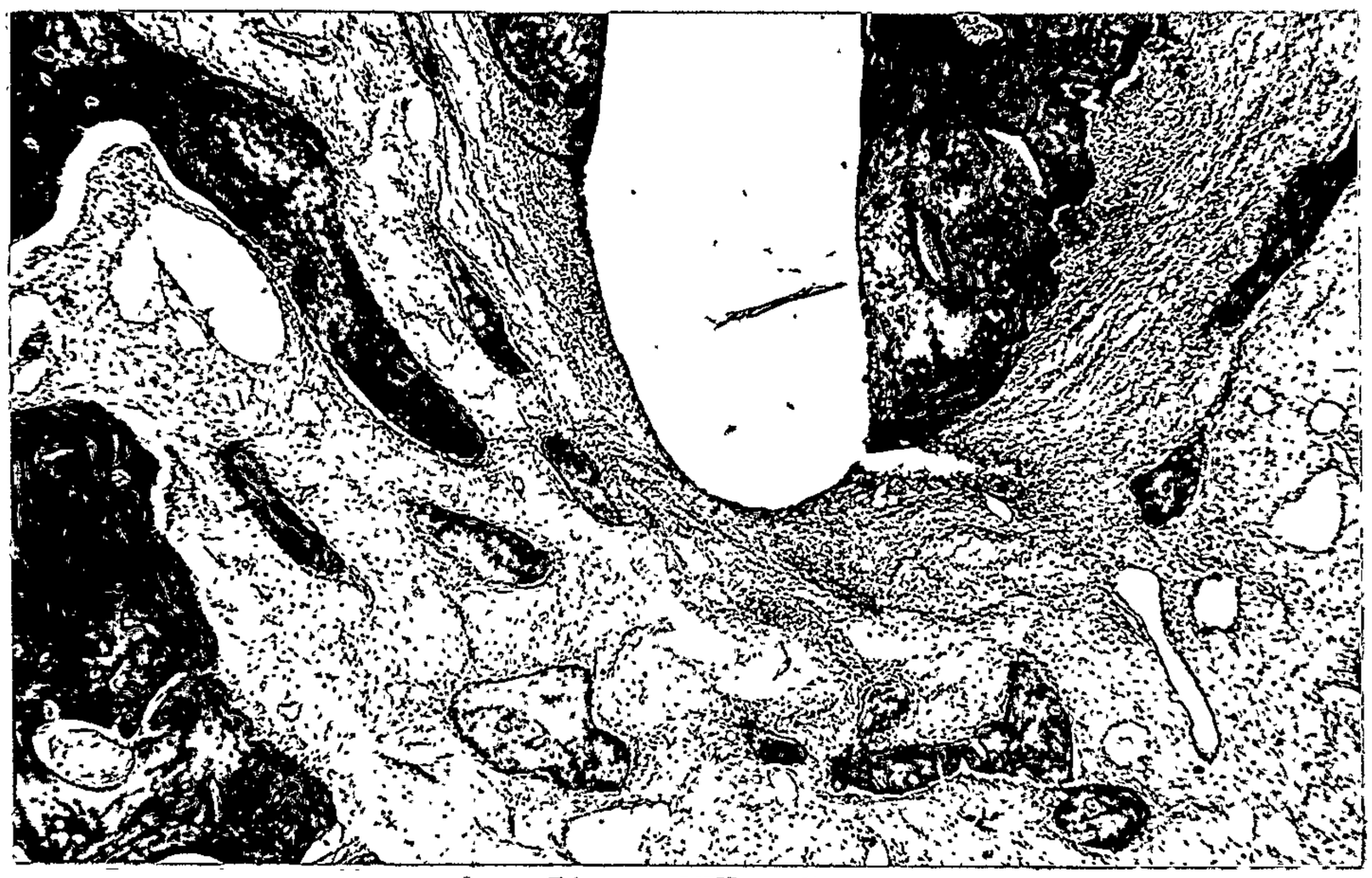

Fig.76 x25

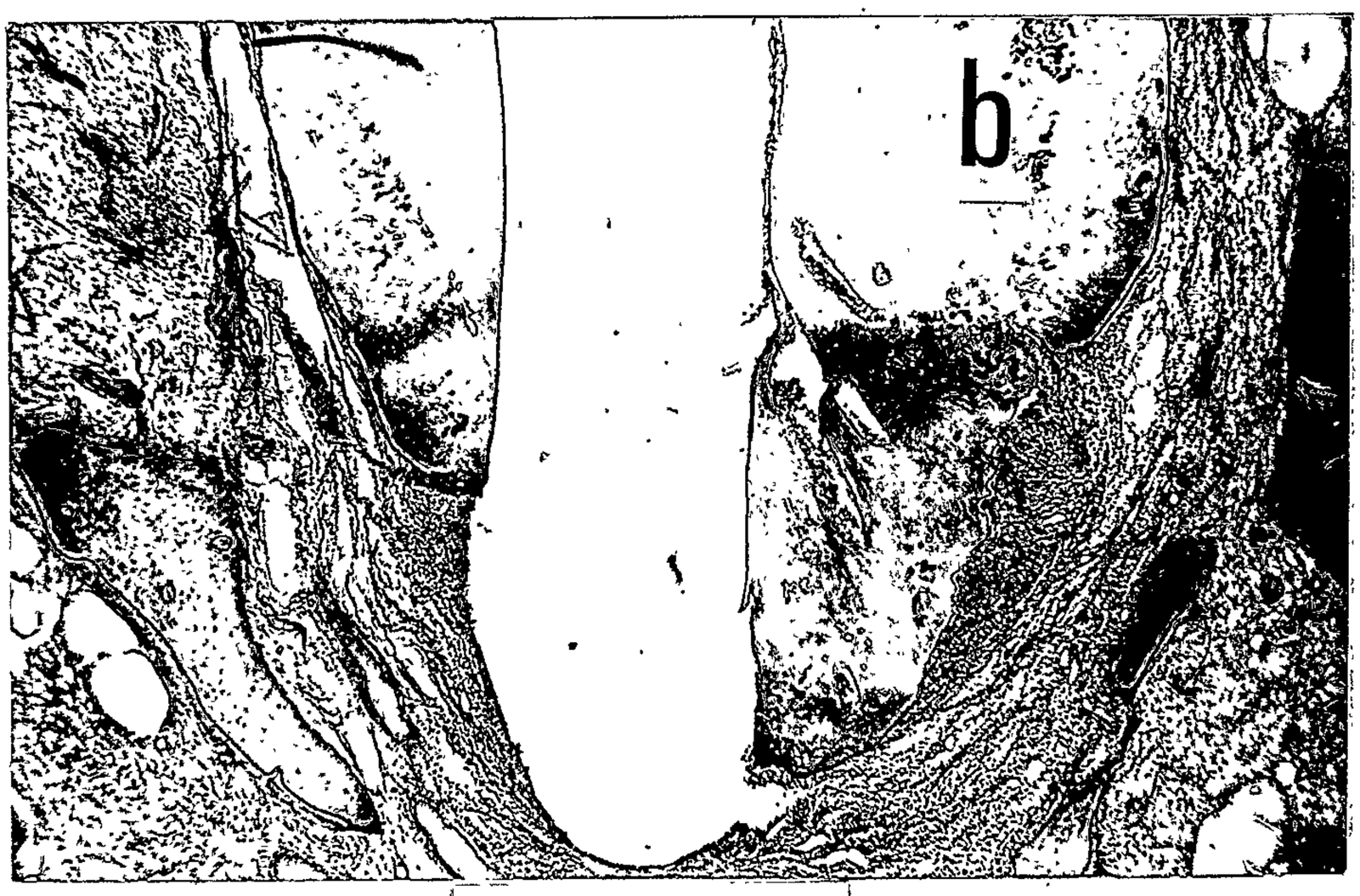

Fig.77 x25
Gram

Experiment 14. Figs. 72 - 77, page 73, 74.

Streptococcus viridans was inserted into each root canal following pulpectomy in the lower left 4th premolar (e). After 3 months, an area of rarefaction had developed on each root apex (fig.72). The mesial root canal was left untreated as a control while the distal apex was perforated, the canal irrigated with sterile water, and immediately overfilled with KRI paste supported by a gutta percha point to the apex (fig.73).

After sacrifice three months later, the radiograph showed the KRI paste to be resorbed from the apical region, leaving the gutta percha point clearly defined (fig.74). The area of rarefaction over the mesial apex was of considerable size, while distally it had been reduced to a slight thickening of the apical periodontal membrane.

The low power photomicrograph (fig.75) revealed the large area of reaction mesially with resorption of the root, while distally the apical area was seen to be filled in with loose connective tissue containing scattered bone trabeculae. A high power view of the distal apex (fig.76) showed fibrous condensation around the slightly extruded gutta percha point and also some foci of inflammatory cells adjacent to apical canal ramifications.

Stained with Gram (fig.77) pockets of bacteria (b) were evident in the cementum of the distal root and in some of the ramifications.

This suggests that if the population of streptococcus viridans is reduced by root filling the main canal, the periapical tissues can effect a considerable amount of repair in spite of residual organisms in lacunae and ramifications. It is possible that KRI paste aided the reparative processes in this situation.

Experiment 15. Figs. 78 - 82, page 76, 77.

In both the lower left premolars shown in Fig. 78, pulpectomy was followed by perforation of the root apices. Mesially in both teeth, KRI paste was extruded into the periapical tissues but no supporting gutta percha point was used. It was the object of this experiment to observe the extent of paste resorption from the canal. Distally, N_2 was forced into the apical tissues, and a separate

M D.

Fig.78

Fig.79

Fig.80 x17

Fig.81 x19

Fig.82 x125

report on the results of this experiment is found on page 210.

After 3 months, the radiograph taken at the time of sacrifice showed the KRI paste resorbed from the apical portion of the root canals (fig.79) and there appeared to be some fragmentation of the paste above the level of resorption.

Histologic examination revealed in each case an ingrowth of connective tissue into the apical part of the canal (figs. 80,81). In the lower left third premolar there was some ingrowth of cementum along the canal wall as well. At the site of fragmentation of the paste seen on the radiograph, there was partitioning by soft tissue, and this connective tissue ingrowth could be traced in serial sections throughout the canal width (figs. 80,81). A higher power photomicrograph showed these fibrous tissue strands to be very cellular.

While healing in these cases must be regarded as satisfactory, it would seem preferable to reinforce KRI paste with a gutta percha point inserted into close proximity to the apex.

Experiment 16. Figs. 83 - 85, page 79.

Bacteria had been inserted into both mesial and distal root canals of the lower left fourth premolar for a period of two months. The distal apex was perforated and KRI paste extruded into the periapical tissues while the mesial canal was left as control (fig.83). After a further 2 months, the paste was seen to be resorbed well up the root canal and some partitioning was apparent in the paste above the level of resorption (fig.84).

Histologic examination of the root apices revealed persistent inflammation around the mesial apex (fig.85) and gram staining showed heavy contamination of the cemental lacunae and ramifications.

Distally, healing had occurred although sparse numbers of bacteria were detected in lacunae. There had been some resorption of the apical cementum with widening of the canal orifice and an ingrowth of connective tissue into the canal lumen. In this case, however, tissue ingrowth did not extend to the level of the resorbed paste.

It must be assumed that the space between the paste and the apical tissue was occupied with fluid which in no way interferred

Fig.83

Fig.84

Fig.85 x11

Fig.86

Fig.87

Fig.88 x9

Fig.89 x32

Fig.90 x22

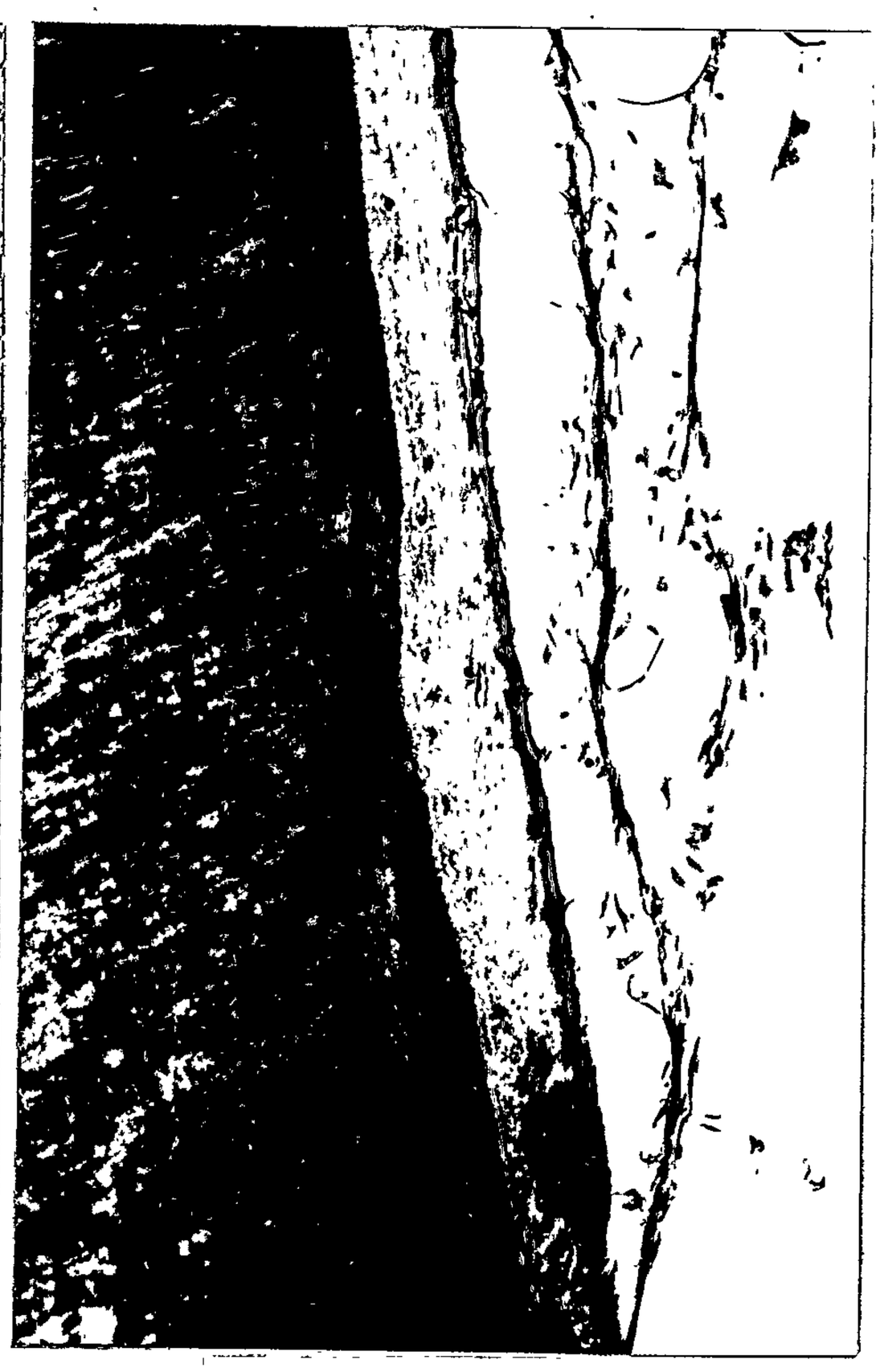

Fig.91 x125

Fig.92 x20

Fig.93 x125

with the apical healing process. The fragmentation of the paste in this case was not due to connective tissue ingrowth.

Experiment 17. Figs. 86 - 93, page 80, 81.

Pulpotomy, followed by application of KRI paste to the exposed pulp tissue, was carried out in the distal canal of the lower right fourth premolar, while immediate root filling with the paste to the cemento-dentinal junction was effected in the mesial canal (fig.86).

The radiograph taken after 3 months showed no change in the level of the paste either mesially or distally (fig.87). The mechanisms which operate when the paste is exposed to the periapical tissues - either dissolution in the tissue fluids and drainage via lymph channels and/or resorption by macrophages - are not operative when the paste is in contact with pulp tissue or is confined to the main canal with only small ramifications communicating between the paste and the periapical tissue.

The tissue juxtaposed the paste in the distal pulpotomy showed some condensation with cellular necrosis (fig.89). The remainder of the pulp was reticular, vacuolated and possibly oedematous although this appearance is reminiscent of artifact due to inadequate fixation (fig. 88). An unusual observation was the cementum-like tissue which lined the walls of the pulp canal in varying depth throughout the apical third of its extent. Low and high power photomicrographs of this tissue are shown in Figs. 90, 91, 92, 93.

Experiments 18, 19. Figs. 94 - 97, page 84.

Views of a lower right second molar are shown in Figures 94, 95 and 96. The pulp had been exposed in this tooth and KRI paste applied with moderate pressure to the bleeding pulp.

The sections shown in Figures 94 and 95 were taken at different levels and reveal that a vigorous secondary dentine response had occurred sealing off the distal part of the pulp chamber from the mesial canal. Figure 96 illustrates the incorporation of dentine chips into newly formed hard tissue.

Figure 97 depicts a lower premolar which had also received a dressing of KRI paste in a pulpotomy wound. In this case also, newly elaborated hard tissue had sealed off the mesial canal from

the coronal pulp and there was marked narrowing of the pulp canal apical to the exposure site.

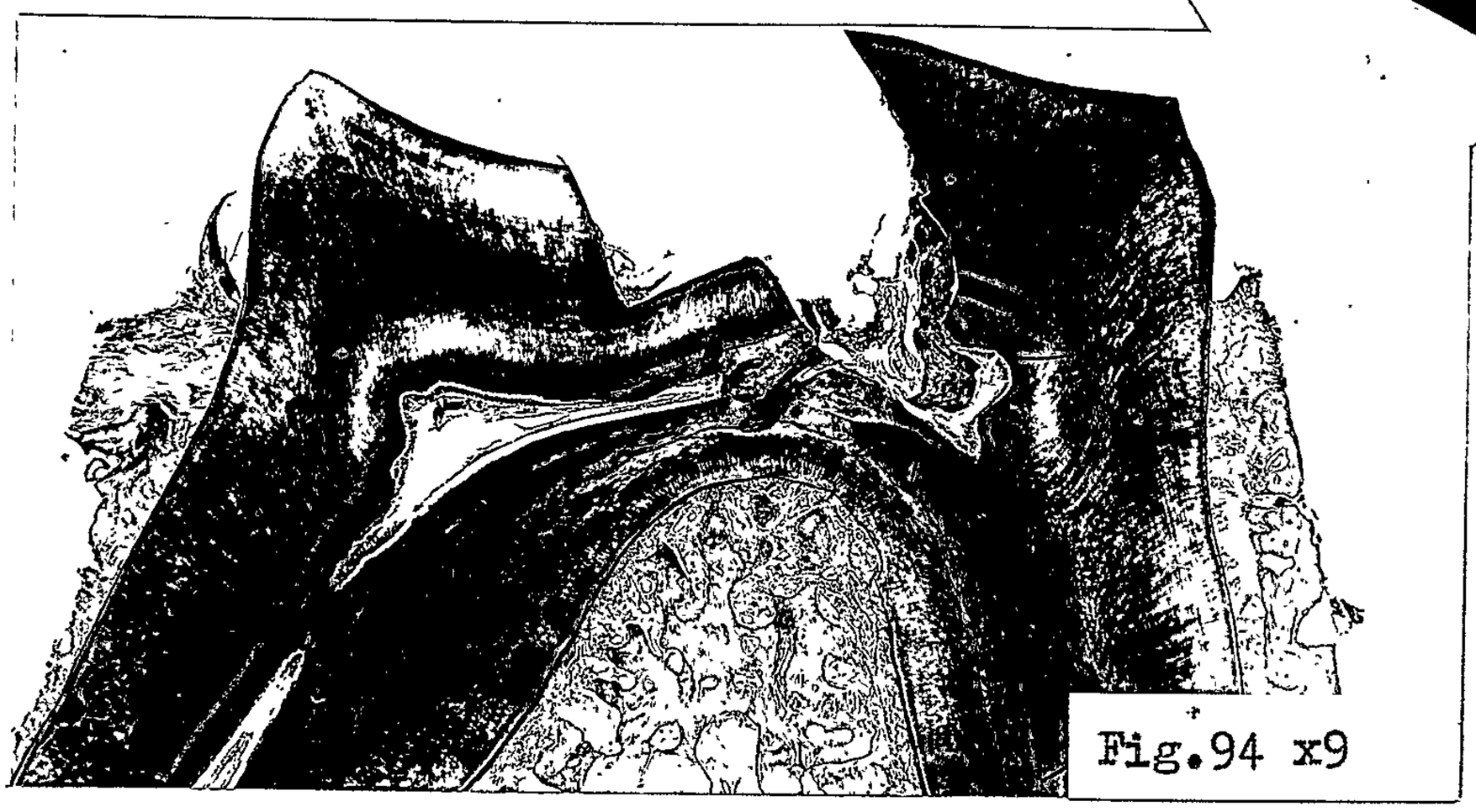

Fig.94 x9

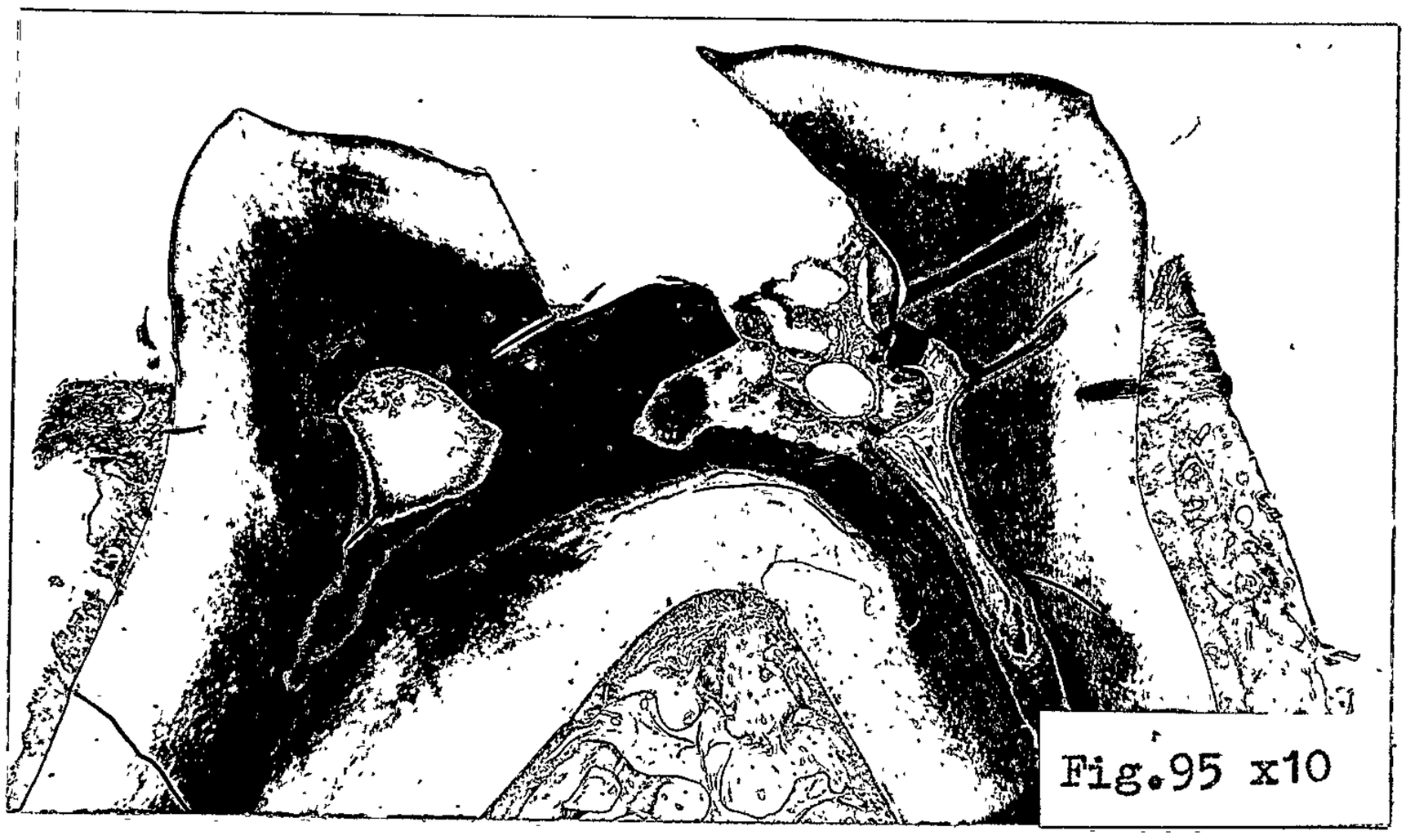

Fig.95 x10

Fig.96 x32

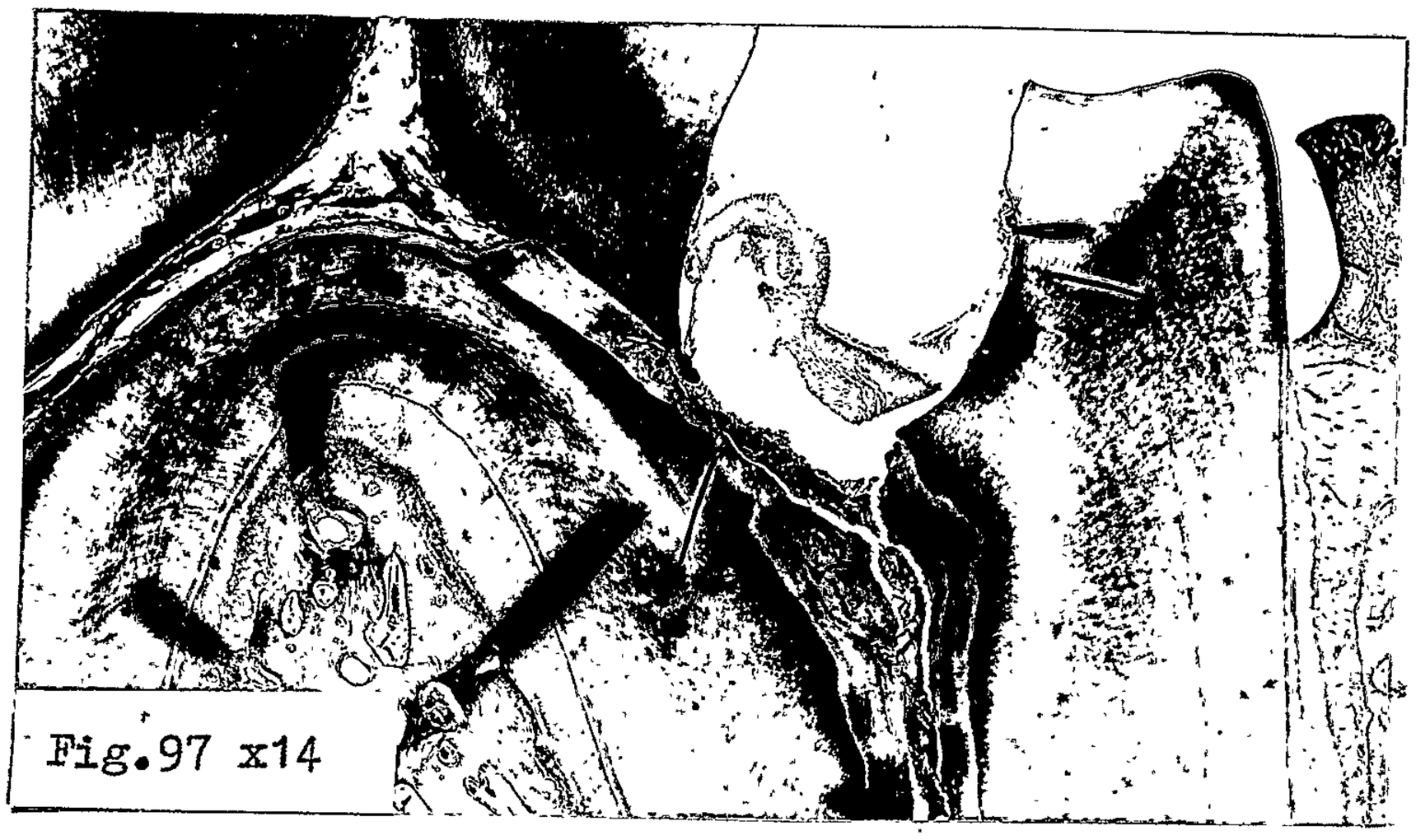

Fig.97 x14

Teeth treated	Treatment	Period of observation	Results
10. LR 3rd Pm with apical areas due to inoculated streptococcus.	Apices perforated and KRI paste extruded into periapical tissues. GP points short of apex. Penicillin for three days.	4 months	Apical healing; paste resorbed from canal and replaced by ingrowth of connective tissue. Evidence of phagocytosis of residual paste in periapical tissue.
11. LR 4th Pm with apical areas as above.	Mesial canal root filled to C.D. junction with sealer and GP. Distal apex perforated and overfilled with KRI. GP point through apex.	7 months	Apical healing around both roots. Denuded GP point had excited some epithelial proliferation.
12. LR 4th Pm. Report on mesial root only. Vital pulpectomy.	Mesial apex perforated; KRI paste extruded together with GP point.	2 weeks	70% of paste resorbed, mainly by direct drainage to lymph nodes. Some evidence of digestion by periapical cells.
13. LL 3rd Pm. Vital pulpectomy.	Canals flooded with streptococcus culture for 10 mins. following apical perforation. Mesial canal overfilled with sealer and GP; distal overfilled with KRI paste with short GP point.	6 months	Healing around both apices; KRI paste resorbed up canal and replaced with connective tissue and cementum. Mesially, connective tissue between sealer and canal wall. No bacteria stained.

14.	LL 4th Pm with apical areas due to inoculated streptococcus.	Mesial control. Distal apex perforated and overfilled with KRI paste. GP point to apex.	3 months	KRI paste resorbed around GP point; apical healing except for small inflammatory foci around canal ramifications. Bacteria persisted in treated apex.
15.	LL 3rd and 4th Pm mesial roots of each.	Mesial apices perforated and KRI extruded. No supporting gutta percha point.	3 months	Resorption of KRI from apical portion of canals. Replaced by ingrowth of periodontal tissue, strands of which had partitioned the paste above.
16.	LL 4th Pm. Bacteria inoculated into canals produced small areas.	Distal apex perforated and KRI extruded. Mesial control.	2 months	Healing distally with resorption of paste to a higher level than connective tissue ingrowth. Fragmentation of paste not due to soft tissue in this case. Sparse bacteria distally.
17.	LR 4th Pm. Vital.	Pulpotomy distally and KRI applied. Mesial canal filled to C.D. junction with KRI.	3 months	No loss of paste from either canal. Distal pulp tissue disorganised but could be artifact; cellular hard tissue had formed on canal walls. Mesial periapex undisturbed.
18,19.	LR 2nd Molar and a lower L 3rd premolar.	Pulpotomy in both teeth followed by application of KRI paste to pulp.	5 months and 6 months respectively.	Secondary dentine response partially isolated paste from vital pulp.

Discussion

These preliminary experiments provide an insight into the singular tissue reaction which occurs following extrusion of resorbable paste into the periapical region, but further investigations are required in order to assess whether the iodoform based material offers any distinct advantages over zinc oxide root sealer as a root canal filling material.

When resorbable paste is extruded through the apex it is completely eliminated from the tissues. Periapical excess of zinc oxide material, however, remains indefinitely, although the foreign body reaction it excites appears to be mild. Indeed, in experiment 13 which compares the tissue response to these two materials following overfilling, normal fibrous tissue insinuated itself between the zinc oxide root filling and the canal wall.

These experiments lend some support to the contention that iodoform paste, when passed into a periapical area of rarefaction, promotes the rapid replacement of the granulomatous tissue with healthy connective tissue, but this impression must be confirmed. In experiments 14 and 16, even though bacteria continued to infect the root apex, the use of KRI paste appeared to excite reparative processes and periapical inflammation was markedly reduced when compared with the infected control apex. On the other hand, healing around both infected apices occurred in experiment 11 and in this case the obturation of one canal with root canal sealer to the cemento-dentinal junction was just as effective as overfilling with resorbable paste. Further experiments which are designed to assess the rate of healing when using these materials would be of value.

Since experiment 11 illustrates that root canal obturation per se allows healing of periapical areas produced by streptococcus viridans, the results of experiment 10, where penicillin was administered parenterally, are inconclusive. All that can be deduced from the fact that apical healing occurred in this case is that resorbable paste, the penicillin and the apical defence mechanisms operated in concert to counteract the bacteria and bring about resolution.

Concerning the mechanism of paste removal from the tissue, Curson[55] and Ostby[185] suggest that both phagocytosis and leaching away into tissue fluid may be the means of paste elimination. Experiments 10 and 12 lend support to the possibility of these dual mechanisms operating. In experiment 10, after 4 months, a microscopic focus of paste was seen to be undergoing phagocytosis. In the case of experiment 12, however, where the tissue was examined after 2 weeks and active elimination of the paste was obviously in progress, few recognisable macrophages were observed around the material although cells in the associated lymph nodes had retained large quantities of black staining foreign matter. It may be tentatively deduced that the initial clearance of the iodoform paste from the tissues is mainly by way of direct drainage to the lymph nodes; residual particles which for some reason remain insoluble are then digested by free cells. When the material is confined to the pulp or unperforated root canal, neither process operates.

Long term studies are required to determine the end result of using unsupported resorbable paste in root canals. Resorption of the material from the apical region does occur and is replaced to a certain extent by ingrowth of periodontal tissue which occasionally lays down cementum like tissue on the canal walls. In experiment 13 this lining of cementum extends further into the canal than the apical soft tissue which suggests that tissue ingrowth had previously extended to a higher level. There also appears to be an upper limit to this tissue ingrowth and this may vary with the diameter of the canal. In two experiments, 13 and 16, paste had been removed to a much higher level than that attained by the apical plug of tissue. Fragmentation of the unsupported paste tended to occur but in two of the three cases studied, this was due to partitioning by connective tissue strands. With the present state of our knowledge, it would seem advisable either to support the paste with gutta percha points or to use a hardening cement in the coronal 2/3rds of the canal.

The use of iodoform paste as a pulp capping material in two cases resulted in the maintenance of pulp vitality with a vigorous secondary dentine response beneath the material. In a third

experiment, newly formed cellular hard tissue resembling cementum was laid down on the canal walls beneath the pulp capping. The pulp appeared to have degenerated, but the technique of perfusion does not necessarily guarantee complete fixation of pulp tissue in all teeth in the animal, and the possibility of artifact cannot be overlooked. It is interesting to note that KRI paste in contact with pulp tissue does not leach away. In the periapical tissues, the material is rapidly surrounded by a dense cuff of small cells which may in some manner, assist in dissolving the paste and aid in its resorption. A similar situation does not appear to develop when the material is superficially applied to exposed pulp tissue.

Rowe[202], who root-filled cats' teeth with various materials to the cemento-dentinal junction, found that in a series of four canals filled with KRI paste, only one possessed normal periapical tissue free of cellular disturbance after 20 weeks. In a single similar experiment using the dog, vital tissue existed in the ramifications apical to the iodoform paste and the periapical tissue was normal.

Further comparisons of tissue reactions in experimental animals are required and until these are forthcoming, extrapolation to the human situation of results gained in any one animal must be made with due caution. The pulp and periapical tissues in the cat may be shown to react in a more exaggerated manner than those in the dog following experimental endodontic procedures. Provided the tissue reaction in the dog is at least as sensitive as in the human, however, valid conclusions in the sphere of clinical endodontics may be permitted from experimental data using this animal.

SECTION 4

The use of Calcium Hydroxide

Although Grove[103] did not think secondary dentine could be deposited where the odontoblasts had been lost or destroyed, Hermann[116] proved otherwise. He showed that a living pulp amputated and covered with Calxyl, a mixture of calcium hydroxide, $NaHCO_3$, $CeCl_2$ and KCl, would repair itself by bridging across the cut pulp with secondary dentine beneath the capping. New odontoblasts appeared beneath the new secondary dentine.

In recent years, calcium hydroxide has been widely used for pulp capping and pulpotomy. It has been used successfully over exposed pulps and after pulp amputations to promote the formation of dentine bridges in the teeth of humans, dogs and rats. Although a good deal of clinical and experimental evidence has accumulated to show that this material causes the formation of a dentine roof more frequently and more quickly than do other substances, the precise mechanism of action is not yet understood.

Zander[254] in 1939 showed complete healing of vital amputated pulps covered with calcium hydroxide and reported 70% success out of 120 teeth using this material.

Glass and Zander[91] compared the healing of exposed young pulps which were treated either with zinc oxide and eugenol or calcium hydroxide. No healing was noted under the former material; the pulps remained vital but a chronic inflammatory reaction persisted. Calcium hydroxide, however, promoted rapid healing relatively free from inflammation and within 4 weeks the exposure site was walled off by a new dentine barrier. (This has recently been challenged by Massler and Miyamoto[154] who found that the pulp is damaged less under zinc oxide and eugenol without calcific bridging than under calcium salts with bridging).

Slack[231] described 26 cases of fractured incisors treated with calcium hydroxide, only two of which failed. Two to three months were required for a bridge to be seen radiographically.

Hess[119] in a review of the European literature states that covering exposed pulps with calcium hydroxide preparations or

following vital amputation of the coronal pulp results in a success rate of 85 - 90%. Covering with zinc oxide and eugenol on the whole gave bad results with 90% failures.

Nyborg[177] confirmed the healing power of the pulp and the pulp tolerance to calcium hydroxide and this was further illustrated by James, Englander and Maesler[128].

Bork[20] reported on 120 cases of pulp capping and pulpotomy studied over a period of one year. All pulpotomies were successful but three cappings failed. He thus confirmed the favourable results of other investigators. In addition he illustrated that the pulps of dogs' teeth were capable of healing in a similar fashion. After 2½ months, six dogs teeth were examined histologically and he shows convincing photomicrographs of the healed pulps under newly formed dentine bridges.

Via[248] however, reports a very high failure rate following pulpotomy and capping with calcium hydroxide in deciduous molars. He studied 103 pulpotomised molars by X-rays for an average period of 24 months, and found a failure rate of 58.9%. Via required the appearance of a dentine bridge for a pulpotomy to be labelled successful. He also noted that in the failure group, 48 teeth exhibited internal resorption with or without periapical involvement. Cabrini et al[37] also reported an incidence of internal resorption of dentine in 28.5% of cases after coronal amputation and treatment with calcium hydroxide. Masterton[156] considers this internal resorption of dentine may be due to lack of healing after pulpotomy with persistent chronic inflammation.

Law[143] carried out 251 vital pulpotomies of which 127 or 51% were failures. He required a normal lamina dura, an absence of spontaneous pain and vital pulps, but he did not insist on a discernible dentine bridge for success.

Shankle and Brauer[223] investigated 70 teeth which had presented vital exposures when capped with calcium hydroxide in methyl cellulose, and found by probing that 52 subsequently came to possess dentine bridges. Age had little influence on the success of the capping procedures and the size of the exposure did not alter the

ability of the pulp to produce a dentine bridge. This is contrary to the advice of Grossman[100] who states that repair can only be expected from a simple exposure of a healthy uninfected pulp.

Masterton[157] however, reported that pulp amputation is a successful operation in the treatment of exposed infected pulps. He described 32 teeth successfully treated by carrying out pulp amputation even though infected. If merely capped with Calyxl, however, exposed infected pulps do not heal. Pulpotomy is necessary.

Calcium hydroxide has also been used for indirect pulp capping. Law and Lewis[144] applied calcium hydroxide paste over residual caries for a 6 month period, followed by complete excavation and restoration by conventional means. A 76% success rate was gained. Of interest was the observation of a radiopaque area pulpal to residual caries which seemed on X-ray to circumscribe the floor of cavity. They were unable to define the cause of this radiopacity.

A mixture of calcium hydroxide with other materials has been attempted, and comparison with other drugs has been investigated by many workers.

Jensen[129] carried out pulp capping in incisor teeth of the dog using calcium hydroxide at a pH of 11.3, or a mixture of calcium hydroxide, zinc oxide, iodoform plus eugenol and phenol at a pH of 7.9. He found a hard tissue bridge had formed in 4 weeks in both instances, and there was no distinguishable difference in the rate of healing or in results produced by either capping agent. He concluded that there was no advantage or disadvantage in adding other ingredients to calcium hydroxide.

Hunter[122] experimented with pulp exposures in dogs' teeth and among other materials used calcium hydroxide and magnesium hydroxide. Of the 10 calcium hydroxide treated teeth, six showed bridging, the remaining four being destroyed by suppuration. Five of the 10 magnesium hydroxide treated teeth showed bridging, the other five failing because of suppuration. The similar dentine stimulating effect of both calcium and magnesium hydroxide may be due to the fact that both possess an elevated pH. He concluded that

the cation was unimportant provided it remains bland. An elevated pH however cannot be more than part of the story concerning the ability of calcium hydroxide to stimulate dentine formation, for other compounds, such as ammonium hydroxide at the same pH cause liquefaction necrosis.

Sekine et al[215] found various combinations of drugs with calcium hydroxide effective and stated that pulpotomy was very successful following the use of calcium hydroxide, sulfathiazol guanofuracin and iodoform.

Seltzer and Bender[217] encountered great difficulty in attaining healing of exposed pulps in dogs' teeth. Mechanical exposures were capped with various drugs - tricalcium phosphate, calcium chloride, calcium carbonate, calcium hydroxide, ammonium hydroxide, alkaline phosphatase and potassium penicillin. Calcium hydroxide was the only calcium compound capable of stimulating reparative dentine formation. However, in some controls medicated with saline, a dentine bridge was formed without the use of drugs. Dentine chips acted as stimulants for the laying down of dentinoid material.

The process of healing under the calcium hydroxide dressing has been studied by many workers.

According to Glass and Zander[91] using calcium hydroxide alone, a sharp demarcation line of basophilic calcium proteinate separates a necrotised zone of injured tissue from the viable tissue soon after operation. In a matter of 14 days after the operation, odontoblasts and a matrix of coarse collagenous fibres which become calcified can be detected below the layer of calcium proteinate and 4 weeks later the odontoblast layer is continuous.

Hess[119] states that after capping, sections show a superficial calcified scar above the exposed pulp beneath which can be seen the formation of new odontoblasts out of the undifferentiated cells of the pulp. Mills[162] considers that new odontoblasts develop from fibroblasts.

Nyborg[177] found the following layers: superficial debris,

the necrotic layer, a blood pigment layer and a dense zone. The necrotic layer soon separates from the subjacent tissues and it is common to see spaces between the necrotic layer and the dense zone. Contact with calcium hydroxide haemolyses blood and gives rise to the blood pigment layer. In the dense zone, vascularisation occurs leading to organisation of the fibrin rich tissue. After 2 days the surface of the dense zone displays necrobiosis and after 7 days calcareous deposits occur deep in the zone.

Berk[19] states that healing following pulp amputation and covering with a calcium hydroxide paste, appeared to occur by organisation of the blood clot, with fibroblastic activity resulting in the formation of a protective callus of organic collagenous tissue. Calcification of this tissue was then noted with entrapped fibroblasts often visible in the irregular dentine. After one month odontoblasts then appeared and regular tubular dentine formation followed. The newly formed dentine bridge is visible on X-ray after 3 months.

Berman[21] and Berman and Massler[22] used zinc oxide and eugenol and calcium hydroxide in maxillary 1st molars of rats following pulpotomy. A stereotyped pattern of healing was observed, the same after 21 days for both materials. This was characterised by the formation of a superficial zone of pulp injury and degeneration under which a layer of dystrophic calcification was formed followed by the building of a true dentine bridge. The blue staining zone of dystrophic calcification was called the primary calcific bridge and was characterised by the appearance of large degenerating pulpal cells entrapped within a dense network of fibrous strands. The primary calcific bridge appeared after 14 days, and the deep blue stain suggested that the area was undergoing calcification. They state that calcium hydroxide is not essential for bridging in view of the occurrence of bridging under zinc oxide and eugenol. However, it caused a more rapid necrosis and degeneration of the superficial layer of pulp tissue and a more rapid appearance of the primary calcific bridge.

Shroff[227] does not consider that calcium hydroxide fulfills the requirements of a true biologic dressing. He states that it is

essentially necrotising in action producing a dystrophic calcification which substitutes for normal repair of the exposed surface.

The mechanism by which calcium hydroxide promotes healing is not understood and there are several theories. In spite of extensive studies it is not yet clear whether the rapidity of its bridging is due to its acid neutralising action - a high pH favours precipitation of calcium salts - to the calcium ion or even to its coagulating powers. Zander believes that calcium diffuses from the calcium hydroxide dressing into the pulp and enters into the formation of a new dentine roof. Others suggest that the calcium for the bridge is derived from the pulp via the vascular supply since good dentine bridging can be observed under dressings which do not contain calcium. Sciaky and Pisanti[222] used radioactive calcium to trace whether diffusion of calcium ions occurs from the calcium hydroxide placed over under amputated pulps into dentine bridges or from the pulps of dogs' teeth. Autoradiographs showed no evidence of radioactivity within the area of new dentine formation and they deduced that the Ca 45 was confined to the dressing. They assumed that the calcium ion was derived from the pulp itself. In 1964[190] the same authors carried the experiment to a further stage and intravenously injected Ca 45 after placing calcium hydroxide over pulp wounds in dogs. Their findings suggested that the calcium in the newly formed secondary dentine comes from the blood stream, for some radioactivity was noticed in the dentine bridges.

However, Stark, Myers, Morris and Gardner[235] used radioactive calcium hydroxide in 24 molar pulps in 4 monkeys following exposure. After 2 months, autoradiographs were made and an intrapulpal localisation of Ca 45 of a sufficient magnitude to present a discernible darkening of the film was seen. This was interpreted as a migration of radioactive calcium from its original area of deposition at the exposure site to a point within the pulp. The mobility of the calcium in calcium hydroxide is therefore not fully clarified from radioactive calcium studies.

Teuchar and Zander[242] propose a theory of the action of

calcium hydroxide. The pH of a saturated solution of this material is about 12 and will readily provide calcium ions. The blood normally contains Ca++ and $PO_4^{\equiv}$ in saturation or super-saturation. Any factor which increases the concentration of either ion will therefore tend to cause the precipitation of $Ca_3(PO_4)_2$ since the solubility product $(Ca^{++}) \times (PO_4^{\equiv}) = K$ will be exceeded. The presence of a large excess of Ca++ provided by the calcium hydroxide therefore encourages precipitation of $Ca_3(PO_4)_2$. The enzyme phosphatase which hydrolyses organic phosphates has an optimum pH of 9.4; the alkaline paste therefore provides a suitable environment for the action of this enzyme. There is a possibility therefore that the application of the paste leads to an enhanced production of inorganic phosphate with increased precipitation of $Ca_3(PO_4)_2$ forming a barrier at the junction of pulp and paste. This precipitate is not organised and does not resemble dentine in composition. Below this seal, biological regeneration of dentine may occur in favourable circumstances. Zander in 1939 stated that the first part of the dentine bridge formed was an amorphous calcified layer which formed a matrix for a regular dentine formation.

Seltzer and Bender[218] consider that the mechanism for the induction of dentine formation under calcium hydroxide may be that it causes a superficial coagulation necrosis of the pulp. Because of its high pH it helps keep the region in a state of alkalinity which is necessary for bone and dentine formation. Under this region which is saturated with calcium ions, cells from the underlying pulp tissue differentiate into odontoblasts which then elaborate matrix.

Castagnola and Orlay[39] also feel that alkalinity of the calcium hydroxide favourably influences the healing of the pulp wound. The pulp has a slight alkaline reaction similar to other healthy tissues. Inflammation results in a shift to acidity and calcium hydroxide neutralises acid by-products which follows an injury and thus facilitates healing. According to some, calcium has a stimulating influence on the odontoblasts. These authors also agree with Teuchar and Zander in considering that the local surplus of calcium should facilitate the sedimentation of $Ca_3(PO_4)_2$ by the

alteration of the factors of the Ca/P solubility product.

Nyborg[177] considers that there may be an initial and superficial precipitate of $CaCO_3$. In other parts of the body, mineral salts are precipitated in necrobiotic tissue and an alkaline environment favours calcification. He states that the mineral salts are transported to the necrobiotic tissue from the pulp and it is not a case of precipitation from the capping material.

Berman and Massler[22] state that the action of calcium hydroxide in promoting healing may be on the basis of its high alkalinity and coagulating action rather than on its calcium content.

Hattyasy[113] regards the capping material as a potential reserve out of which, according to the conditions for solution and to the flow of blood, calcium hydroxide can become soluble. In the presence of CO_2 the formation and precipitation of $CaCO_3$ occurs, dependent on the molecular concentration of the calcium hydroxide and the nature of the organic matrix. Many experiments have shown that other capping materials cannot compare with the dentinogenic potency of calcium hydroxide so that in addition to its alkalinity it appears to have a specific activity.

Masterton[158] reasons that the first stage of dentinogenesis is the formation of a basement membrane consisting of argyrophil fibres from the pulp which are arranged parallel to the junction. This process of basement membrane formation occurs before, and entirely independent of the differentiation of the odontoblasts. It is suggested that the necrotic layer produced by the calcium hydroxide acts as an organiser surface or induction factor for basement membrane formation and that consequently it assumes the functions of ameloblasts. In addition it probably protects the underlying tissues while they form the more durable calcific scar.

Fish[85] is very cautious about the success of pulp capping and pulp amputation. In these operations, if a small infected fragment of dentine is displaced into the pulp it will form a necrotic nidus in which organisms can grow indefinitely causing a chronic or acute abscess. On the other hand, capping an exposed pulp in an animal under aseptic conditions can be done with impunity. Any dentine

fragments which fall into the pulp, being sterile, will in part be resorbed and then covered over with callus, that is, with the usual type of calcified repair tissue that the pulp produces. Many chips may thus be welded together forming the bulk of the dentine bridge. Seelig[214] considers that it is possible for a dentine bridge to form at one point while the pulp has a localised abscess caused by infected dentine chips in another.

Encapsulation of dentine chips by callus was only occasionally noticed by Kalnins and Frisbie[131] who commonly found localised or diffuse pulpitis developing around chips from carious cavities. They also state that in cases of circumscribed pulpitis of low severity, a dentine bridge was formed above the focus of inflammation. This could lead to cases of advanced pulpitis even when the radiographs show dentine bridge formation.

Masterton[158] states that clinical examination for a barrier is a valuable method of determining whether pulp healing has been successful. If the hard tissue barrier is complete, he considers the pulp is vital and healthy below it. This is not agreed to by other authors, and cases of pulp degeneration under a dentine bridge have been reported.

Seltzer and Bender[218] state that in spite of bridge formation, the pulp sometimes remains chronically inflamed and eventually succumbs. The formation of a bridge is not the final proof of successful pulp capping or pulpotomy. The formation of a bridge does not guarantee success or its absence presage failure. Zander[254] describes a case where a lower molar had a pulpotomy performed 7 months before extraction. The dentine bridge was well developed but the pulp had entirely disintegrated, and only necrotic tissue remnants were found. He was unable to determine why one case should turn out favourable and another one fail.

Experiment 20. Figs. 98 - 101, page 101.

A radiograph taken at the time of operation shows the mesial canal of the lower left 2nd premolar (a) continuous with the coronal pulp chamber following exposure and capping with calcium hydroxide (fig.98). Six months later, the radiograph shows a dense barrier intervening between the mesial canal and the pulpotomy channel (a) (fig.99).

Serial sections revealed that a dentine bridge had been erected and this was continuous throughout, providing a complete barrier beneath the calcium hydroxide (fig.100). Higher power photomicrography illustrated the typical irregular dentine which occurs in a case of successful healing, and in this case some chips of primary dentine were enclosed (fig. 101).

The pulp was disorganised, and it is possible that this was artifact due to inadequate permeation of fixative during perfusion. It must be noted, however, that this degeneration of the pulp was observed in three cases where a hard tissue barrier had been formed under calcium hydroxide, and pulp tissue in adjacent teeth, similarly perfused with fixative, was not disorganised.

Experiment 21. Figs. 102 - 106, page 102.

Pulp capping with calcium hydroxide was carried out in the lower left second premolar (fig. 102). After 3 months, a dentine bridge was discerned on the radiograph circumscribing the pulp wound (fig. 103).

Serial sections showed that the bridge was complete and was formed mainly of atubular dentine encasing a large number of dentine chips which had been dislodged into the pulp at the time of operation (figs. 104,105). As in the former case the pulp appeared degenerate in the coronal portion and only remnants remained. The possibility of artifact must be considered. A photomicrograph of the apical portion (fig. 106) showed vital pulp tissue and the periapical tissues were intact.

Experiment 22. Figs. 107 - 112, page 103, 104.

The lower right second premolar in Figure 107 received a calcium hydroxide dressing following pulpotomy in the mesial canal.

Nine months later, a very radiopeque line was observed on the radiograph deep to the pulpotomy wound (a, fig. 107) and this line was far more radiopaque than usually seen following eraction of a normal dentine bridge.

The lower left 4th premolar depicted in Figure 108 (b) was similarly treated. In this case, calcium hydroxide had been in contact with exposed pulp tissue in the mesial canal for a period of 2 weeks only, prior to sacrifice. By comparing the radiograph taken at the time of operation (fig. 108b) with that obtained at the time of sacrifice (fig. 109b) it became apparent that a dense radiopaque area of some magnitude had developed deep to the pulpotomy wound during the two weeks. A further radiopaque line was seen lining the floor of the pulpotomy cavity.

A section of this area (fig. 110b) showed a necrotic layer deep to the pulpotomy wound coinciding with the position of the radiopaque area. A high power photomicrograph of this strongly basophilic zone suggested that it was composed of a dense conglomerate of necrotic tissue which has presumably absorbed calcium salts (fig.111). Further down the canal in the region of a denticle was an haemorrhagic area (fig. 112) beyond which lay normal pulp tissue. Whether a normal dentine bridge would have eventually formed beneath the haemorrhagic zone in the manner described by Nyborg[133] is impossible to forecast. It is equally possible that further pulp degeneration might have occurred, or alternatively a walling off of this area with connective tissue may have supervened.

A search of the literature does not reveal any report which describes a similar event. The marked radiopacity in this experiment was not produced by a dentine barrier but by a plug of necrotic tissue which must have become heavily impregnated with calcium salts.

Experiment 23. Figs. 113 - 116, page 106.

The mesial pulp horn of the lower left 4th premolar was exposed and a calcium hydroxide pulp cap inserted. Distally the pulp had been removed and a paste of calcium hydroxide in distilled water was used to fill the canal to the cemento-dentinal junction. A short gutta percha point was used to condense the paste into the apical portion of the canal (fig. 113).

Fig.98

Fig.99

Fig.100 x12

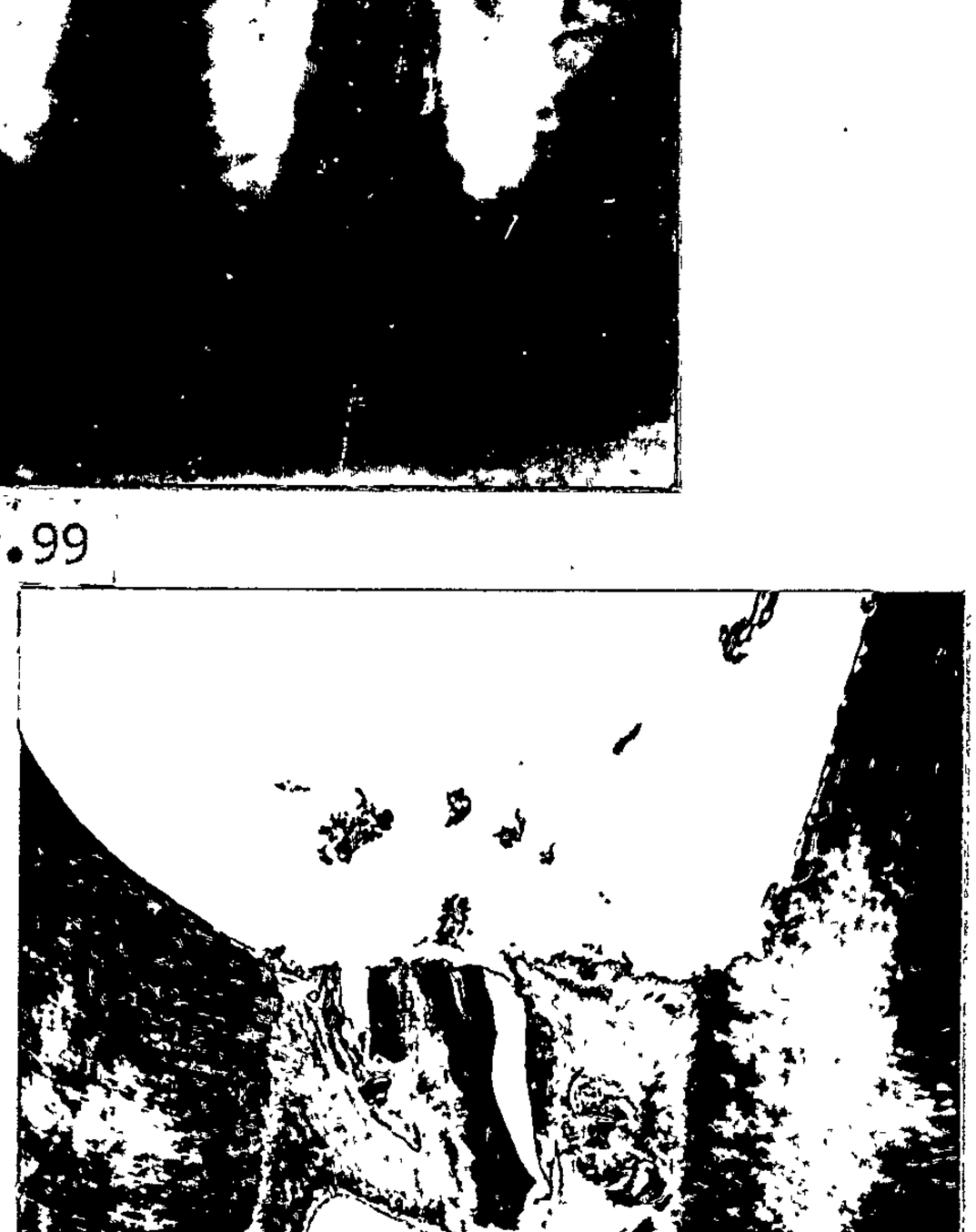

Fig.101 x32

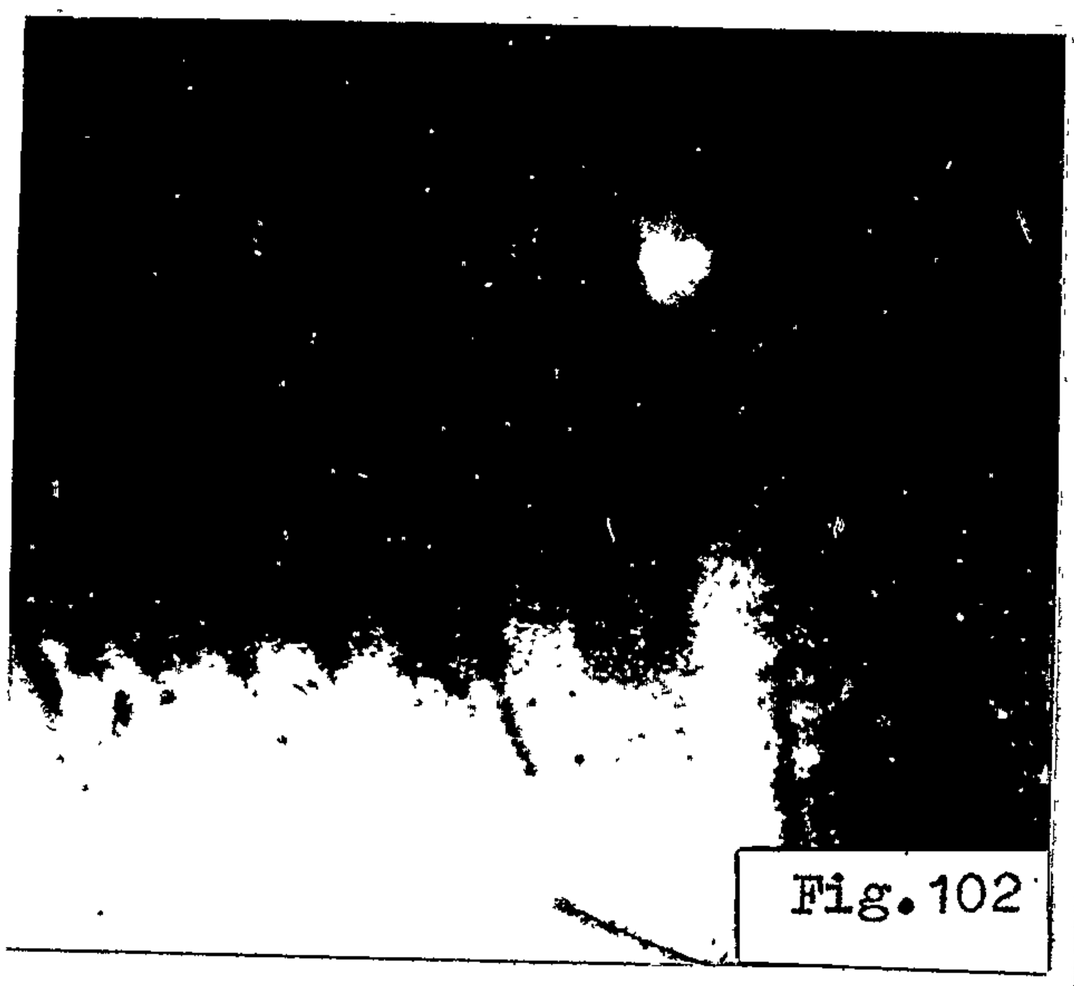

Fig.102

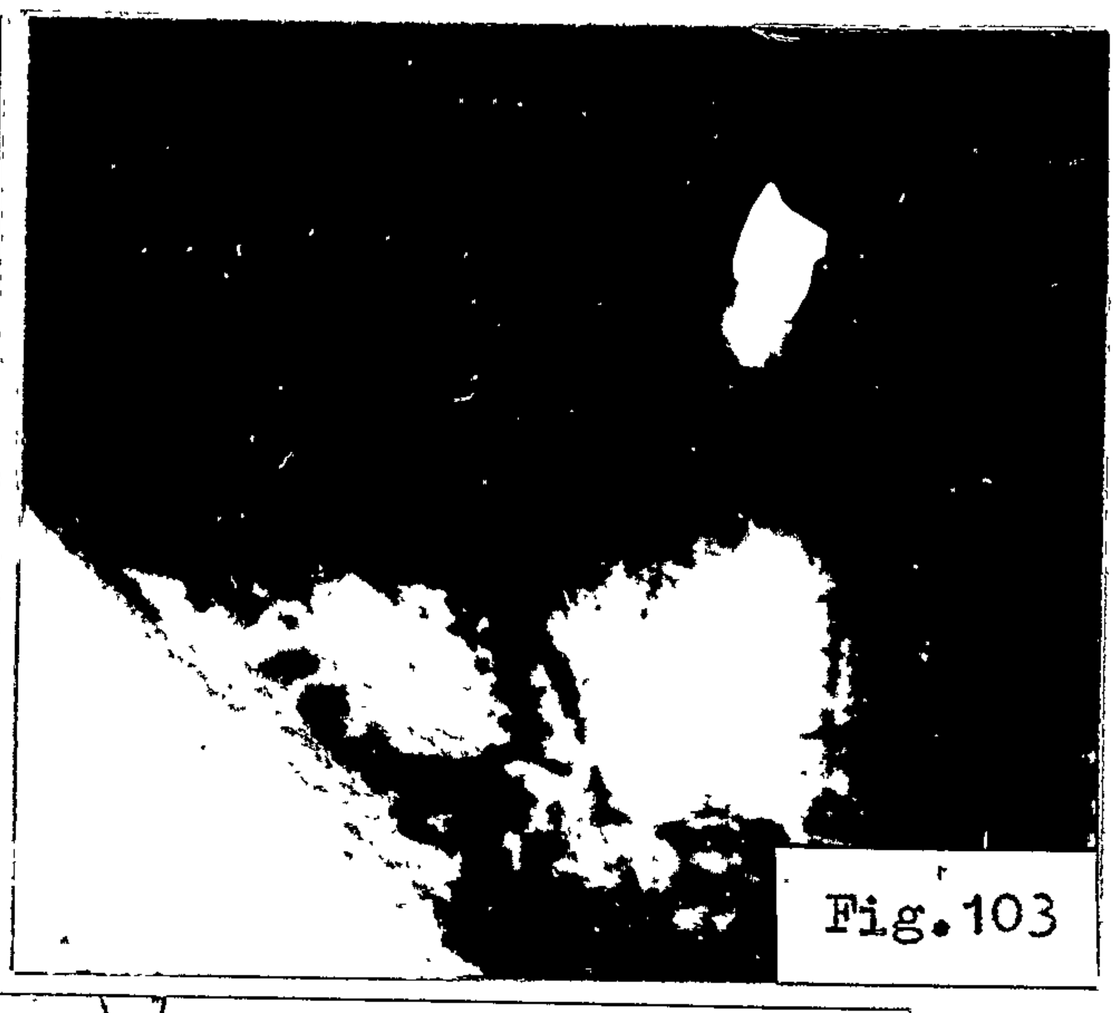

Fig.103

Fig.104 x12

Fig.105 x12

Fig.106 x18

Fig. 107

Fig. 108

Fig. 109

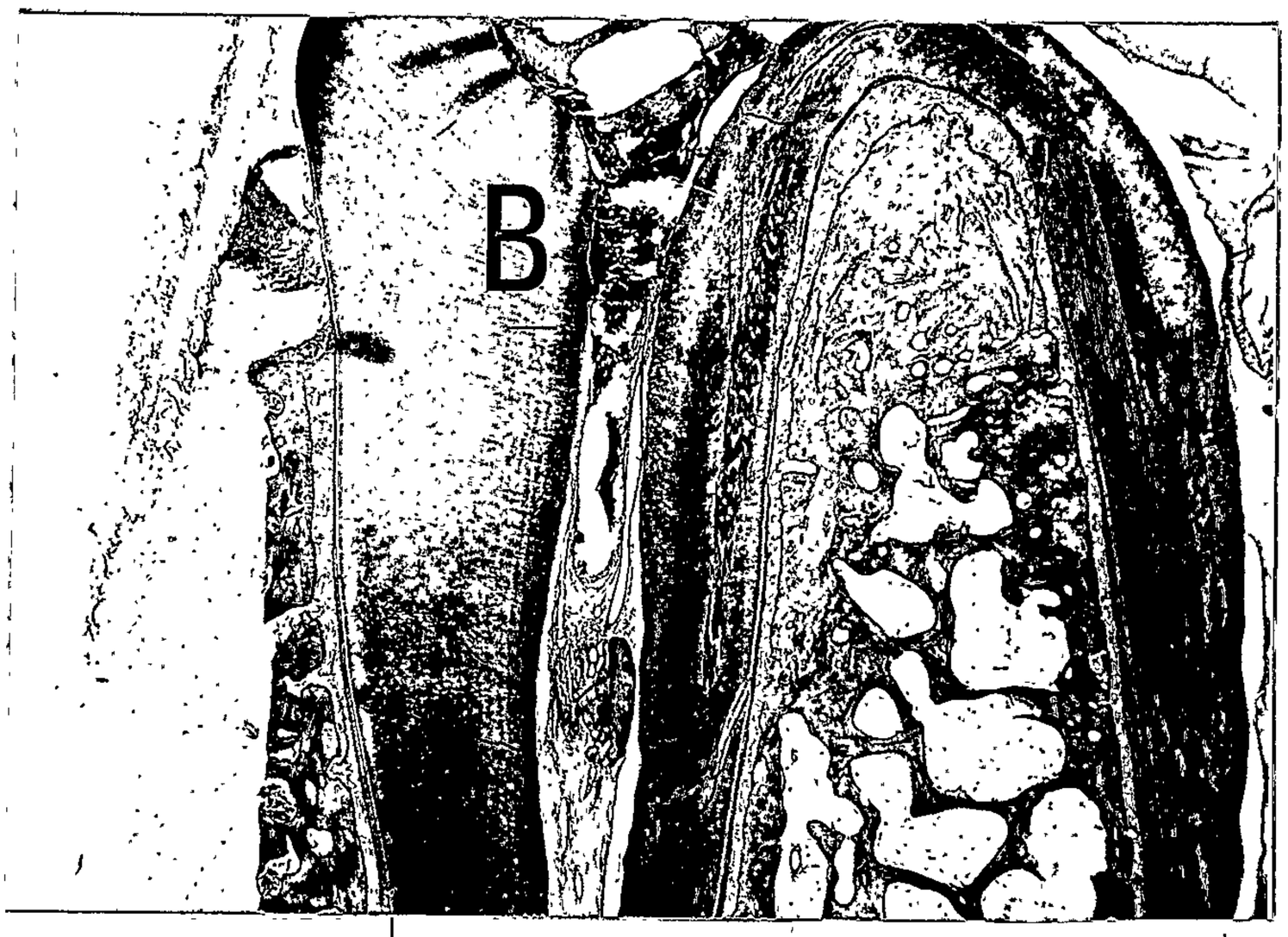

Fig.110 x10

Fig.111 x125

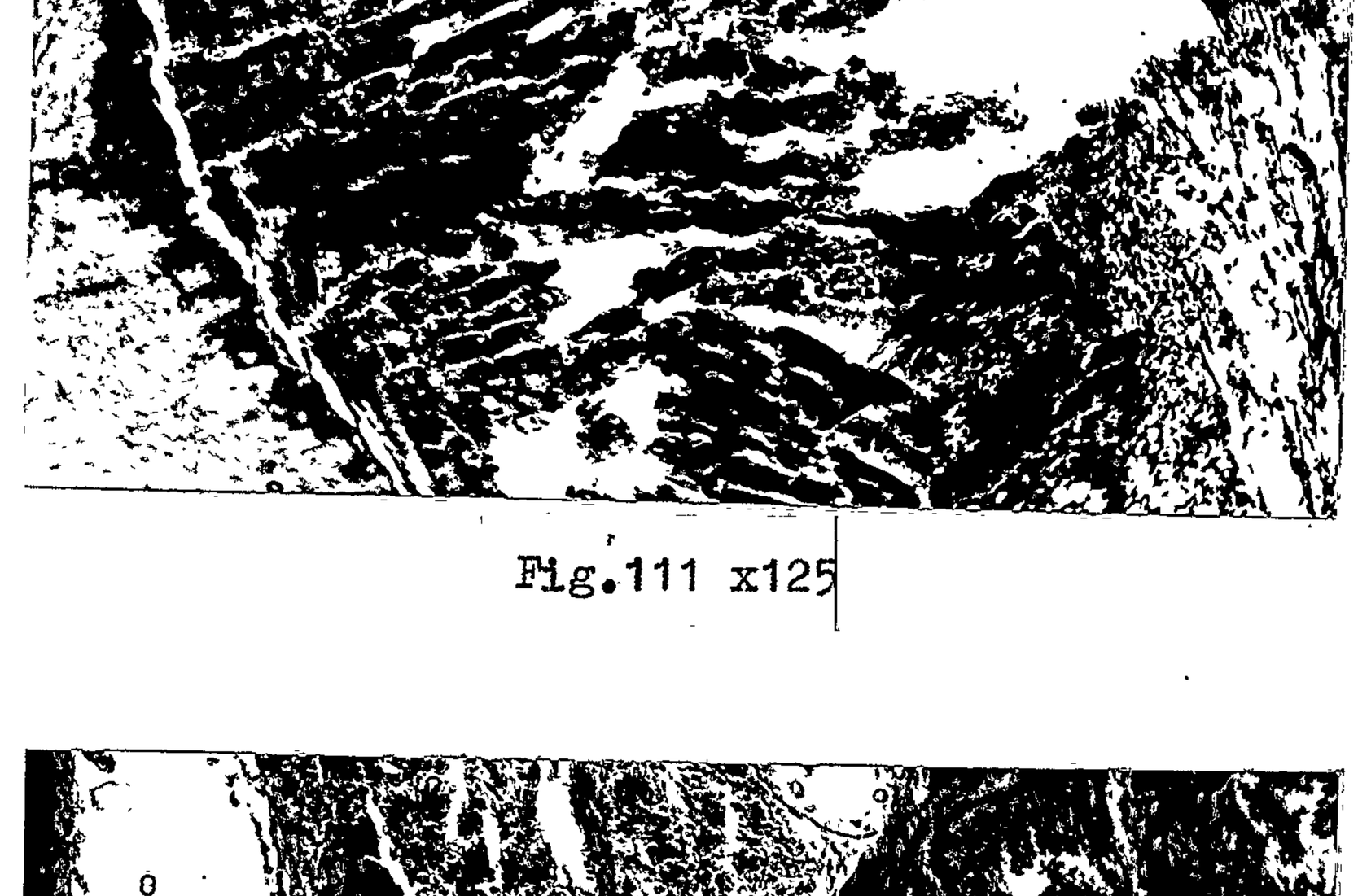

Fig.112 x125

At the time of sacrifice 6 months later, a radiograph revealed a radiopaque plug in the mesial canal deep to the area of pulp capping (fig. 114 a). As in the former case, this was subsequently observed in histologic section to be an aggregation of necrotic tissue and no hard tissue barrier had been formed (fig. 115). Whether the calcium salts which had impregnated the necrotic tissue were derived from the overlying calcium hydroxide or carried to the site by the pulp tissue is not clear. The remainder of the pulp appeared degenerated, but this may have been artifact due to inadequate fixation rather than the end result of treatment. The periapex was sound (fig.116). It is of interest to note that the calcium hydroxide root filling had caused some periapical reaction. This was a chronic response, localised to the immediate vicinity of the root apex.

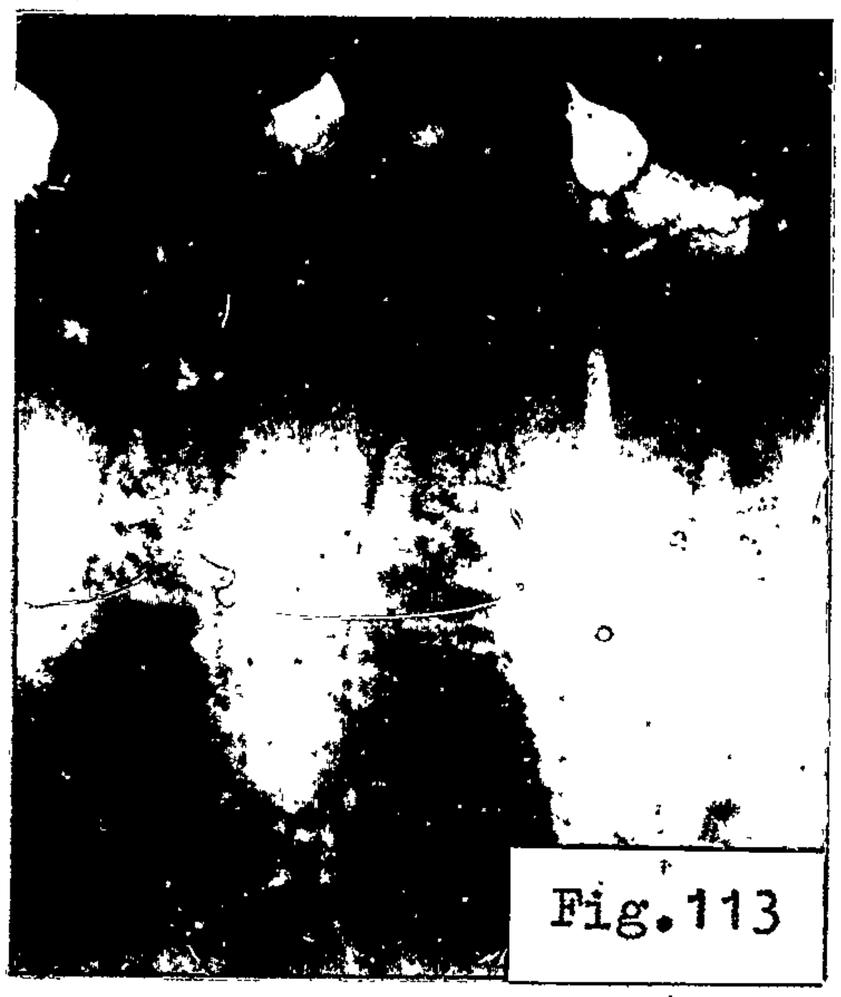

Fig.113

Fig.114

Fig.115x20

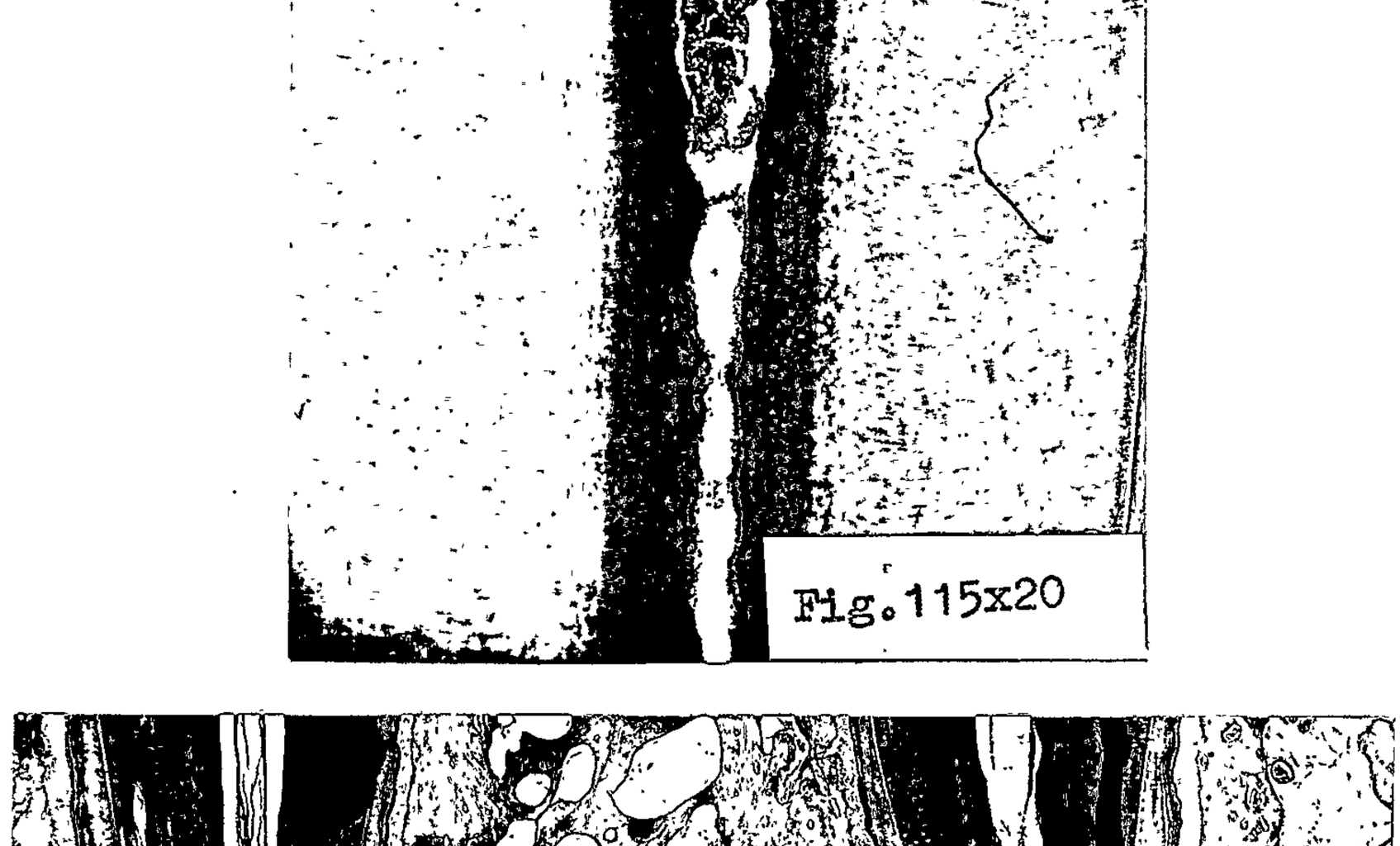

Fig.116 x10

Tooth treated	Treatment	Observation period	Results
20. LL 2nd Pm	Mesial pulp capping with calcium hydroxide	6 months	Complete dentine bridge formed. Pulp degeneration (? artifact)
21. LL 2nd Pm	Pulp capping with calcium hydroxide	3 months	Intact dentine bridge. Pulp degenerated in coronal portion. (? artifact)
22. LR 2nd Pm and LL 4th Pm	Mesial pulpotomy in each case followed by calcium hydroxide capping.	9 months and 2 weeks respectively.	Dense radiopaque line beneath pulp cap in each case. This corresponded to a dense plug of necrotic debris. No dentine barrier.
23. LL 4th Pm	Mesial pulp cap with calcium hydroxide. Distal root filling to cemento-dentinal junction with calcium hydroxide.	6 months	Mesially, a dense plug of necrotic debris beneath pulp cap had caused a dense radiopacity on radiograph as above. Distally, chronic inflammatory reaction apically.

Discussion

These experiments reveal that the application of calcium hydroxide to dog pulp results in a variable and sometimes quite atypical response. In some instances a dentine bridge is formed and this may be no more than atubular callus cementing together masses of dentine chips. In one case reported, the bridge was composed of irregular secondary dentine and was similar in every feature to dentine bridges occurring in human teeth (expt. 20).

Degeneration of the pulp beneath the calcific bridges occurred in each case but the possibility of artifact due to inadequate fixation has been referred to. It must be noted, however, that Zander[254] reported pulp necrosis in a human tooth following elaboration of a dentine bridge and further investigations are required to assess the incidence of this phenomenon. If serial sections reveal an intact bridge, as occurred in the dog material studied, it seems unlikely that pulp degeneration could have been caused by constant irritation from the calcium hydroxide.

In three cases studied, a unique response to calcium hydroxide was observed. No hard tissue barrier was formed, but an area of cellular degeneration had occurred deep to the wound surface. This dense zone had absorbed calcium salts, was strongly basophilic and was almost completely radiopaque. It is possible that a similar result has been observed radiographically in the human, incorrectly diagnosed as a dentine bridge. A dentine bridge has the same radiographic density as surrounding tooth structure however, and this may serve to distinguish a true hard tissue barrier from the densely radiopaque plug of necrotic debris. There are no reports in the literature concerning this latter response to calcium hydroxide and a critical reappraisal of post-treatment radiographs showing 'bridging' in human teeth appears warranted.

Calcium hydroxide is currently being used by some clinicians as a preliminary root filling material to encourage periapical healing and to promote calcific repair at the apex prior to sealing the canal with non resorbable material[55]. Frank[87] used calcium hydroxide

and camphorated para monochlor phanol in root canals to aid the closure of open apices, and Michanowicz[161] recommended placing a layer of calcium hydroxide at the apex with root canal sealer and gutta percha filling the remainder of the canal. Kennedy et al[133] state that passing the material beyond the apex is of no consequence as it is well tolerated by the tissues.

Limited animal experimentation does not support the contention that calcium hydroxide is bland when in proximity to the apical tissues. In experiment 23 reported above, root filling with calcium hydroxide caused chronic inflammation periapically. Rowe[202] used the cat for similar experiments and after examining the effects of a wide range of materials, he concluded that calcium hydroxide in distilled water produced the most severe reactions of all. Future experiments in which dog root apices are perforated would provide a means of studying the tissue reaction to calcium hydroxide when used as a root filling.

SECTION 5

Glucocorticosteroid/antibiotic materials

The treatment of exposed pulps and inflamed periodontal tissues with a combination of a glucocorticosteroid and an antibiotic has been the subject of several reports in the literature during the past 10 years. An infected pulp has difficulty mounting a full inflammatory reaction without injuring itself in the process, a consequence of the rigid confining walls of the pulp chamber, the lack of collateral circulation and the pressure of the inflammatory exudate. The application of the glucocorticosteroid/antibiotic compound to a pulp in the early stages of inflammation is aimed at reducing the intensity of the reaction by suppressing the cellular exudate and inhibiting the proliferation of micro-organisms, so that resolution may eventually occur.

One of these materials in widespread use is Ledermix, of which the principal ingredients are triamcinolone acetonide and demethylchlortetracycline. It is marketed in two forms: a water soluble cream containing only the corticosteroid and antibiotic, and a rapidly hardening cement. The water soluble cream is recommended for the treatment of acute pulpal conditions, as it continues to liberate its active ingredients as long as it remains over the site of application. The rapidly hardening cement is based on the zinc oxide/eugenol formula and contains the same active ingredients in a smaller concentration. It is recommended for use as a lining in deep cavities to allay pulp reaction following operative procedures and for the permanent capping of carlously exposed and hyperaemic pulps which have initially been treated for a few days with a dressing of the paste. Calcium hydroxide was added in the hope that it might stimulate dentinogenesis but there is no evidence that it is effective in such low concentration. It is also possible that it is neutralised by the acid tetracycline hydrochlorides.

Many clinicians attest to the effectiveness of this compound in rapidly relieving the pain of pulpitis and some reports indicate that a large number of the treated teeth continue to respond to vitality tests after long observation periods (~~1-8~~) (212, 199, 167, 71, 72, 179, 180, 1). Positive response to tests however does not

necessarily denote a healthy pulp, and the long term result of using these materials on inflamed and infected pulps is still uncertain. If sterilisation of the pulp cannot be effected, then eventual necrosis under the cement capping will occur, albeit clinically silent. Harris[109] as well as Gotze[94] have shown by means of fluorescence studies that when the cement is placed over the exposed pulp, the tetracycline component spreads rapidly throughout the pulpal tissues to the apical region and its effect probably persists for some time. This widespread diffusion of the antibiotic may affect many of the infecting bacteria, but as was pointed out by Ehrmann[72], not all the organisms in the cariously involved pulp are sensitive to tetracycline, and all yeasts are resistant. Moreover, since the tetracycline is not bactericidal but only bacteriostatic, the defence mechanism of the pulp continues to be of importance and should not be completely suppressed.

In addition, it is a matter of speculation whether the tetracycline can effect sterilisation of infected dentine fragments dislodged into the pulp. Unless these nidi of bacterial growth are eliminated or sterilised, the pulp will not heal and the capping procedure is valueless. In this connection, it must be pointed out that Gotze[94] has shown that tetracycline was not able to penetrate carious dentine. It is perhaps significant that Schroeder[210] recently suggested indirect capping wherever possible, leaving some carious dentine over the pulpal floor provided there is no partial purulent pulpitis. He states: 'An exposure of the pulp due to extensive carious involvement ..... is always a handicap, rendering the prognosis for maintaining tooth vitality dim'.

Fiore-Donno and Baume[84] consider that the corticosteroid compounds are contraindicated for direct pulp capping and medication of pulpitis in permanent teeth, and should be restricted to capping deciduous pulps, temporary relief of painful pulpitis in permanent teeth pending extraction or root canal therapy, and root canal medication, when pulp necrosis is accompanied by periapical complications.

It is appropriate to review the literature tracing the development of Ledermix and related compounds for the treatment of pulp and periapical pathology.

Treatment of periodontitis with corticosteroids.

The earliest use of corticosteroids in endodontics was by Wolfsohn[252] in 1954 who used hydrocortisone in 79 root canal treatments in an attempt to evaluate its effectiveness in the control of secondary periodontitis. It proved to be effective provided the pulp canal had been rendered sterile by electrosterilisation prior to its insertion. Wolfsohn warned that in the presence of infection, this hormone does not seem to be effective for it appears to produce exacerbation of the disease process. He also noted an increased inflammatory response when he used the hormone on an infected pulp.

Parenteral administration of hydrocortone together with antihistamine and oral penicillin was the subject of a report by Stewart[237] who administered these drugs in order to reduce postoperative sequelae following root resection. He further recommended this therapy prior to operating on root canals which were responsible for acute exacerbations, for it usually allowed instrumentation, medication and closure at the first visit, thereby eliminating the necessity for a period of drainage.

With regard to topical application of corticosteroid agents, Blitzer[29] in 1956 used hydrocortisone combined with antibacterial agents and hyaluronidase in the root canal for reducing inflammation in the periapical tissues, and in 1961 Feinschneider[80] employed hydrocortisone plus neomycin in an ointment base for a similar purpose.

Tomasek et al[243] in 1963 reported that a paste combining hydrocortisone and an antibiotic is more effective in the treatment of certain acute forms of periodontitis than is the ordinary treatment with antibiotics alone. The use of the mixed paste usually led to rapid clinical improvement and the disappearance of symptoms. This paste was successfully used on 100 anterior teeth.

By 1963 Schroeder and Triaden[208] had showed that triamcinolone, a far more potent drug than hydrocortisone, when combined with chloramphenicol or demethychlortetracycline in a water soluble base, was a superior mixture for treating acute apical periodontitis. Immediate closure of the canal after debridement and filling with this

paste was advocated even in the presence of acute symptoms. The reaction is stated to recede within the space of a few hours and in the second sitting, the canal is usually secretion free, allowing continuation of root canal therapy by standard methods.

Schroeder[209] uses Asphalin, a formaldehyde preparation mixed with Ledermix paste 1:1 for sterilisation of the canal. He considers nascent formaldehyde still one of the best root canal disinfectants. The irritant action of the trioxymethylene paste is balanced by the anti-inflammatory action of the corticosteroid.

Rowe[202] observed the periapical response to root filling teeth in cats with various compounds and found that a combination of zinc oxide, barium sulphate and cortisone acetate resulted in periapical disturbances in all cases. When cortisone acetate was combined with zinc oxide and paraform however, there was no inflammatory reaction and this result gives support to Schroeder's formula. He found however, that Ledermix cement and paste did not prove to be effective anti-inflammatory agents when used in root canals, for in each case studied, inflammatory cells were seen in the root canal and periapex. These results will be compared with dog studies reported in this section.

<u>The treatment of pulpitis with corticosteroids.</u>

Rapoport[193] in 1958 experimented with vital exposed pulps which were relatively asymptomatic with no apparent pulpitis or hyperaemia. Hydrocortisone acetate was used either in saline suspension or as a powder. In a total of 60 teeth, 93% remained vital 6 months later in one series, while in a second series 80% retained vitality. This second series included old exposures and pain cases. Checks over longer periods were not carried out.

Tuvell and Morales[246] in 1958 described their capping experiments with a paste consisting of six parts of calcium chloride and one part of cortisone acetate in anhydrous glycerine. The experiments were performed on various animal teeth as well as on 16 healthy human teeth which were extracted 25-45 days later. Histologic examination showed the pulps to be healthy; in isolated cases, hard substance formation was observed.

Kiryati[134] in 1958 examined the effect of hydrocortisone alone and in combination with polyantibiotics upon experimentally damaged and infected dental pulps in 1st upper molars of rats. Treatment of lacerated pulps with hydrocortisone alone showed 22% complete healing. When the hormone was combined with a mixture of oxytetracycline and chloramphenicol, a significantly higher incidence of complete healing was observed - 63%. Combination with neomycin and bacitracin however, resulted in only 18% healing. Lacerated pulps covered with oxytetracycline and chloramphenicol without hydrocortisone gave 35% complete healing. These results are inconclusive except to indicate that a combination of hydrocortisone and some antibiotics increased the survival of pulps after injury and infection.

Galluzo and Bellomi[90] in 1959 were the first investigators who attempted to heal serous pulpitis in man with a corticoid/antibiotic mixture. They combined penicillin, streptomycin, tetracycline with prednisolone in a lanolin - vaseline ointment base. They obtained favourable results in 19 cases, after observing teeth over a period of 6 months.

Kozlov and Massler[137] in 1960 investigated the action of a large variety of drugs on the amputated pulps of rat molars. They state that, in rat molars, many materials which are not injurious permit reparative dentine bridges to be formed. Cortisone and hydrocortisone were among the substances which elicited a satisfactory reparative reaction, although in view of the several other successful substances, they point out that the bridging over the pulp does not depend upon the anti-inflammatory effect of the corticoid. It is significant, however, that the glucocorticosteroid does not suppress this calcific response in the rat.

Fry et al[89] in 1960 employed a paste composed of camphorated parachlorophenol, metacresyl acetate and prednisolone soluble. In cases of pulp hyperaemia following cavity preparation, the paste was applied to the crown preparation or the cavity and restored with zinc oxide and eugenol. When a vital operative pulp exposure occurred under aseptic conditions, the paste was applied over the exposure followed by restoration with zinc oxide and eugenol with asbestos sheet between. Also in pain cases due to carious exposure, the paste was

applied, after partial caries removal, to the unexcavated carious dentine. All patients stated that pain subsided promptly and after 4-6 weeks the treated teeth responded normally to vitality tests. They conclude that there is much to be gained by the topical use of such a paste for the relief of dentinal and pulpal pain and for conserving the vitality of exposed pulp tissue.

Triadan and Schroeder[211,244,245] record several cases. They found they were unable to relieve pulpitic toothache by applying 2% hydrocortisone ointment and resorted to the use of triamcinolone, combining it with chloramphenicol and a 4% xylocaine solution in an ointment base. 214 teeth were treated and pain disappeared after 2-3 hours. All showed normal responses to vitality tests and the majority were still vital after some months. In cases of purulent pulpitis, the pain ceased but there was no resolution. It was later found advisable to replace the ointment after one week with a hardening cement containing the active substances in lower concentration. Schroeder states that the corticosteroids are without doubt suitable for suppressing inflammation of the pulp and hence for providing immediate pain relief, but adds that a broad spectrum antibiotic must be added for the pulp to have a chance to survive. He considers that the use of corticoids for capping the healthy pulp seems to be contra-indicated for it brings the pulp from a normergic to an energic condition which is likely to end in necrosis.

Vigg[249] in 1964 used hydrocortisone 1% and oxytetracycline 3% in the form of Terracortril ointment and applied it to exposures. He covered the paste with several medicaments - calcium hydroxide in methyl cellulose, zinc oxide and eugenol, zinc phosphate cement and then a temporary restoration. He reported success in 14 out of 18 pulpitic teeth after 6 months, together with the formation of a calcific bridge in all cases.

Prader[191] in 1962 made a haematopulpogram from 56 pulpitic pulps by making a smear of the first drop of blood following exposure of the pulp. The wound surface was then dressed with a water soluble salve containing a highly active antibiotic substance and a glucocorticoid and the cavity temporarily closed. After 1-5 days a

second smear was made. In 43% of the cases the inflammation and infection were diminished or eliminated, while in 26% the inflammation alone, and in 13% the infection alone diminished. He concluded this treatment was very efficient for treating pulpitis.

Fiore-Donno and Baume[83], 1963, treated 190 teeth with painful pulpitis by direct capping with a variety of corticosteroids including triamcinolone in combination with chloramphenicol. Clinical response to treatment was remarkably uniform, for all painful pulps responded with immediate cessation of symptoms. Extracted teeth were sectioned but favourably clinical results were not corroborated histologically. There was an absence of a solid barrier, and metaplastic fibrosis of the pulp paired with stunted or arrested dentine formation was observed together with residual or induced chronic inflammation. In 1966, Baume[14] warned that this persistent chronic inflammation may cause pulps to succumb without symptoms even after years.

Mosteller[169,170] established the effectiveness of a corticosteroid solution (prednisolone) in eliminating thermal sensitivity under gold restorations in all but 3 of 726 teeth. In 25 mouths, both sides received similar restorations. Only one side was treated with the solution prior to cementation. After 1 week none of the treated teeth were sensitive to water at 46°F while 50% of the untreated teeth were sensitive. Histological sections showed that teeth treated with prednisolone still demonstrated pulpal inflammation but to a lesser degree than the untreated controls. Thus prednisolone does not completely eliminate inflammation in response to deep cavity preparation and Mosteller further considered that the camphorated parachlorophenol in the solution may aid in the desensitising action.

In 1964, Dachi et al[59,60] reported on the use of prednisolone to dull thermal sensitivity and pulpal reactions under silicates and amalgams and found it to significantly more effective than sorbitol placebo. Swerdlow et al[241] also reported a favourable reduction in pulpal reactions following prednisolone therapy. Sayegh[205] et al applied a mixture of prednisolone, parachlorophenol, metacresyl acetate

and gum camphor to cut dentine with sound pulps under class V cavities then restored the teeth with zinc oxide and eugenol. The clinical and histopathologic findings 1-4 weeks later suggested that the steroid formula modified the pulpal response in limiting pathologic change.

Stanley et al[234] using the same solution stated that the formula has definite efficacy in reversing an established focal pulpitis due to trauma of cavity preparation.

Lawson and Mitchell[149] in 1964 carried out a double blind study treating painful pulpitis with a control of starch or a corticoid/ antibiotic mixture of erythromycin, streptomycin and flurandrenolone in starch. Fifty cases were treated and the mixture appeared to have definite therapeutic advantages. No clinical failures were recorded with the corticoid mixture whereas approximately half the control teeth failed. Limited microscopic study showed that clinical findings and histopathologic observations did not always correlate well, indicating that some of the so called clinical successes ultimately may prove to be failures.

In a further report by these authors[171] when clinical testing of treated teeth had been extended for an average of 21 months there had been a fall in the success rate from 100% to 71.4% thus confirming their prediction.

A note of warning concerning the application of glucocorticosteroids alone to exposed pulps, was sounded by Klotz et al[135] in 1965. These authors, using monkeys, found a systemic bacteraemia in 21% of cases whose pulp exposures were treated with streptococcus faecalis and prednisolone. Combination of the corticoid with a broad spectrum antibiotic appears mandatory when treating carious exposures. The work of Klotz should be contrasted with the fact that no bacteraemia has been demonstrated as a result of endodontic procedures confined within the canals or the pulp chamber when no steroids have been used.

The advent of Ledermix

Schroeder and Triadan carried out several clinical investigations using the combination of triamcinolone and a broad spectrum antibiotic chloramphenicol and obtained a high rate of success. Using the combination of Ledercort (triamcinolone) and Ledermycin (demethylchlortetracycline) in 1962[212] they reported on the

treatment of 484 teeth. Rapid subsidence of pain was generally noted. Of the 419 teeth with pulpitis, only 4 were non-vital upon follow up examination 21 days later. With such a short observation period however, no conclusion could be made about the maintenance of vitality. Among the 65 cases of periodontitis, there were three treatment failures. These authors state that the addition of calcium hydroxide produces rather massive deposits of hard substance after a few months.

The Ledermix preparations were thus formulated and to the chlortetracycline and triamcinolone in the cement was added zinc oxide and calcium hydroxide.

Since its introduction, there have been many reports attesting to its value in the treatment of pulp involved teeth, periodontitis and sensitive dentine following cavity and crown preparation.

Rowe[199] in 1963 found all but 9 out of 245 pulp capped teeth retained vitality and were symptomless for a period of 4-8 months after treatment.

Mohammed[167] presented clinical data on a total of 192 patients - 115 with pulpitis and 77 with periodontitis, 66% of which would normally have been recommended for extraction. The use of Ledermix resulted in rapid relief of pain. This was accompanied by maintenance of vitality and lack of radiographic evidence of periapical pathology in 89.5% of the teeth up to 6 months after therapy.

Ehrmann[71,72] in 1964 reported that in deep cavities and crown preparations covered with Ledermix, thermal sensitivity was greatly reduced. In pulp exposures treated with the material, there was an absence of postoperative pain, and in hyperaemia and early pulpitis, the pain ceased and the majority of pulps remained vital. Ehrmann points out the need for histologic studies to determine the state of the pulps after treatment. He also noted the relief from painful pericementitis after using Ledermix. Five exposed pulps were reopened some time after treatment, and while all were symptomless and vital, no bridge was formed. He considers that it cannot be expected that a pulp damaged as a result of a severe inflammatory process will recover sufficiently to lay down secondary dentine. The best to hope

for is an atrophic organ.

A recent communication from Ehrmann[73] reveals that over 50% of the teeth treated in 1964 have since succumbed. This emphasises the need for long observation periods before assessing the results of treatment. Robertson[198], reporting on the progress of 80 treated exposures after 6 months, stresses that his preliminary assessment must be followed up in further studies. Treatment with Ledermix was effective in relieving pain and preserving functioning symptom free teeth in a high proportion of cases.

Whether or not a hard tissue barrier is formed is a matter of some controversy. Schroeder seems to be the only investigator who has demonstrated a complete dentine bridge[213]. More frequently the picture seems to one of narrowing of the exposure site by hard tissue formation, but rarely complete closure. Because of doubts about the formation of a dentine barrier, Schroeder[210] recently suggests indirect capping where possible, leaving some carious dentine over the pulpal floor provided there is no partial purulent pulpitis. Treatment of deep cavities is recommended in two sittings - Ledermix paste followed by either calcium hydroxide or Ledermix cement.

Another deficiency in the Ledermix preparations appears to be the limited antibacterial properties of the tetracycline component. Zeldow and Ingle[255], working on infected root canals, found 24.3% of staphylococci and 33.3% of enterococci resistant to tetracycline. Goldman and Pearson[92] found 23% of organisms isolated from root canals resistant to tetracycline. It therefore seems that Ledermix cannot be relied upon to sterilise the infected root canal and it is improbable that organisms in an infected pulp can be totally eradicated by topical application of the substance.

Ehrmann[72] reported a pilot study showing that out of 19 carious cavities tested, 5 cavities contained bacteria resistant to tetracycline.

Olsen[179,180] in two articles reports encouraging results from the treatment of a total of 722 teeth with Ledermix. These included cases of pulpitis with pulp exposure; hyperaemia without exposure, and periodontitis. Relief of pain was rapid in the majority

of cases. He examined 298 teeth affected by pulpitis 6 to 12 months later and loss of vitality was observed in only 3 cases. In four teeth, microscopic examination was carried out. One case showed an absence of inflammation, two had a pocket of chronic inflammation and showed loss of odontoblasts, and one revealed a cystic area in the pulp. He states that sections suggest closure of the wound with hard substance, but he does not appear to mention that in arriving at this conclusion he had actually examined serial sections.

Laws[148], after treating three cases of carious exposure with Ledermix paste and cement, found no evidence of calcific bridging after periods up to 194 days, but noted an increased fibrosis of the coronal pulp with a tendency for the unaffected pulp to be walled off by a transverse arrangement of fibroblasts and collagen fibres. Diffuse infiltration of the coronal pulp with chronic inflammatory cells persisted in each case.

Allwright et al[1] in 1966 treated vital exposures with Ledermix, some with symptoms of pulpitis. In 34 teeth, painful symptoms subsided in about 2 hours and all remained vital 3-16 months later. They observed no dentine bridge radiographically, but consider that the almost uniformly good results are sufficiently encouraging to warrant wider use of the corticosteroid/antibiotic preparation.

In 1966 Cowan[39] published a report of a clinical trial and the results of microscopic examination of some treated teeth. In the group of 126 teeth treated for carious exposure, 114 or 90% were successful judged by maintenance of vitality. He also selected 9 carious teeth which were causing severe pain and treated the exposures with Ledermix cement only. All became symptomless and appeared to remain vital. They were extracted 14-43 days after treatment and prepared for histological examination. A study of these nine teeth showed that the inflammation had not been controlled with Ledermix although it was possible that the predominately chronic response was an effect of treatment. There were signs of acute inflammation still present especially around dentine chips and suppuration was present in many of the pulps. There was no formation of secondary

dentine. Cowan considers that a vital apical pulp was probably responsible for the positive vitality tests. He concluded that control of pain does not connote successful capping and many pulps undergo necrosis without symptoms over a variable period of time. Ledermix, states Cowan, is useless except as a medium for temporary relief of pain in advanced acute pulpitis and of very limited value in advanced chronic pulpitis. It must be pointed out that the teeth were treated for comparatively short periods, and the possibility that preliminary treatment with Ledermix paste may have caused a more pronounced diminution of the inflammatory reaction was not investigated in this study.

Maeglin et al[152], as a result of an investigation of 159 teeth followed for 12-14 months, considered that there was no resolution but merely a masking of symptoms. Acute conditions were simply converted to a chronic state. In this connection, Schroeder commented: 'Corticosteroids are able to interfere with acute inflammation but are powerless against chronic inflammation'.

Apart from the controversy which exists concerning the usefulness of Ledermix in the treatment of pulp pathology, there are few reports in the literature describing the long term response of normal exposed pulps to this agent.

Fiore-Donno and Baume[84] mechanically exposed thirty pulps with a history of spontaneous pain together with 10 pulps of intact teeth and capped them with Ledermix paste and cement. There was complete remission of symptoms in all painful teeth. After extraction, following postoperative periods of 38-156 days, the pulps were microscopically examined. There was evidence of arrestment of dentinogenesis at the site of exposure and serial sections showed that bridging never occurred. They state that irreversible metaplastic changes of the pulp tissue were observed at the site of contact with the cement which extended to variable depths. These changes comprised atrophy of odontoblasts and fibroblasts and disappearance of Von Korff's argyrophil fibres. Signs of residual chronic inflammation became more prominent as the postoperative period lengthened. This would suggest a gradual diminution of the anti-inflammatory effects of the material. These results are in accord with previous observations

made by these authors using other corticosteroid containing compounds but they state that Ledermix appears to induce reactions that are more localised and less severe. They consider that these corticosteroid compounds are contraindicated for direct pulp capping and medication of pulpitis in permanent teeth.

Major and Ostby[165] in 1966 studied the effect of Ledermix on the normal pulps of recently erupted teeth. In some cavities, either Ledermix cream or the cement was applied to the pulpal floor. In a series of twenty exposures, cement was used to cover the pulp. Observation periods extended from 3 to 177 days and calcium hydroxide was used as a control in some contralateral teeth. When Ledermix was applied to the pulpal floor of cavities, the changes in the underlying pulp were localised mostly in the odontoblast/predentine area, usually without involvement of the rest of the pulp. In some cases when Ledermix cement was used as a lining material it was difficult to discern any histologic changes in the pulp. The first change seen, especially under the paste, was increased basophilia in the cytoplasm of odontoblasts under the tubules exposed by cavity preparation. More marked changed were a localised reduction in the odontoblast layer both in cell height and number. The predentine zone was hardly noticeable or absent.

With pulp exposures, only slight changes were observed in the soft tissue. Reduction of odontoblasts and the predentine layer were seen near the exposure. Sometimes a marked deposition of irregular secondary dentine at a short distance from the wound was found, and the upper portion of this deposit was separated from the primary dentine by trapped cells. Dentine fragments at the level of the predentine zone, where it reappeared, possessed an unmineralised matrix on the pulpal aspect. Fragments near the would caused no reaction. In one instance a fusion of several dentine fragments was observed with an inclusion of cells between the fragments. No dentine bridge was found underneath the Ledermix cement. The marked reduction of the odontoblast layer is considered to be a result of cell destruction and inhibition of dentinogentic activity; the absence of predentine tends to substantiate this. It seems as if the predentine present at the start of the experiment had mineralised and no new predentine

formation had occurred.

Ulmansky and Langer[247] reported on the effect of Ledermix on normal pulp tissue following pulpotomy. Dentine bridge formation had not occurred after eight weeks, but a dense fibrous band had formed separating the pulp from the surface material. This observation is similar to that described by Lawe[148]. Ulmansky and Langer conclude that Ledermix induces a unique type of degeneration, the extent of which is in direct proportion to the total time of action.

Other reports concerning the action of Ledermix on vital pulp tissue do not indicate that pulp degeneration occurs. Cellular reaction appears to be minimal, and when a change is noted it appears to be confined to the area immediately beneath the cement.

Clarke[42] in a histological study of normal pulps after capping with Ledermix cement could not find any pulp changes that could be directly attributed to the capping agent and considered there was no contraindication to its use in clinical pulp therapy.

Harris and Bull[108] treated nine normal pulps with Ledermix cement and recovered the teeth 23 to 314 days later. In eight teeth atubular calcification was found along portion of the walls of the root canals and on the floor of the pulp chamber. While dentine bridging did not occur at the site of the exposure, some zones of amorphous calcific material were observed beneath this region in six teeth. Strongly basophilic amorphous calcified areas were frequently present in the radicular pulp and occasionally in the coronal area. These investigators found few changes in the pulp tissue but drew attention to the presence of large and numerous venous sinuses in all their specimens.

Barker and Ehrmann[11] studied 12 teeth in which Ledermix cement had been applied to normal pulps for periods ranging from 29 to 727 days. Pulp tissue in contact with the cement was vital and apart from some localised reduction or loss of the odontoblast layer and a tendency for the vessels subjacent to the exposure to be dilated, the cement exerted no deleterious effect on the soft tissue. A complete bridge of dentine was not formed in any case studied, although partial closure of the exposure with secondary dentine occurred in three cases. In

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five other teeth, all single rooted, the lumen of the canal beneath the exposure site was narrowed by deposition of a circular collar of aberrant dentine on the canal walls. This tissue was atubular, but possessed deeply staining basophilic streaks and sometimes exhibited lacunae and cellular inclusions. One case of extensive internal resorption occurred around the exposure site.

In addition to the study of normal pulp response to Ledermix cement, these investigators examined three pulps which had exhibited symptoms prior to treatment. Two teeth which were not obviously cariously exposed had been treated with Ledermix cement and symptoms of hyperaemia were alleviated. After 653 days one of these teeth showed a persistent focus of chronic inflammatory cells in the coronal pulp associated with internal resorption, and the other, extracted after 80 days, showed advanced pulp degeneration. A small carious exposure in one molar tooth, causing symptoms of pulpitis, was treated with Ledermix paste for one month. Complete resolution occurred and the pulp tissue was vital in contact with the dressing. This single case illustrates that resolution and healing may occur following use of Ledermix paste on a carious exposure, but the authors point out that the actual disease state of the exposed pulp was unknown at the time of treatment. Baume[13] describes a case of a tooth that presented with all clinical signs of pulpitis, yet on histological section showed only a small degree of hyperaemia and stasis without any other sign of inflammation. Out of a total of 200 teeth which were examined both clinically and histologically a definite correlation could only be established in one third of the cases. Similar observations have been made by Mitchell and Tarplee[164], Hardnt[107] and others.

Barker and Ehrmann concluded that the application of Ledermix cement to normal human pulp tissue does not impair vitality and evokes a moderate calcific response after a prolonged period of contact. When applied to a minimally inflamed pulp, the possibility exists that Ledermix will effect resolution in a proportion of cases, but the outcome of such treatment is impossible to predict. Relief of symptoms and the continued positive response to vitality tests may well conceal

a chronically inflamed degenerate pulp which will ultimately become necrotic. Further long term studies both clinical and histological are required in order to assess the usefulness of this material.

Experiment 24. Figs. 117 - 127, page 127, 128, 129.

The distal portion of the coronal pulp was exposed in the lower right second premolar and Ledermix cement applied with moderate pressure (fig.117). After sacrifice 4 months later, and histologic processing, most of the pulp was observed to be normal.

The odontoblast layer was well defined along the walls of most of the pulp cavity but absent in the area immediately coronal to the exposure (fig. 118) and also in the canal underneath (compare the distal canal in Fig. 120 with the mesial canal in Fig. 121). The predentine layer was not traceable in the area coronal to the exposure and it appeared as if it had progressed to complete calcification in spite of the loss of odontoblasts (fig. 118). The area immediately below the cement presented a reticular appearance and large vacuolated cells were present (fig. 119).

Dentine chips were seen in the tissue deep to the exposure site ('a' in fig. 120; 'e' in fig. 124). Many of these had become encased in cementum and this cementum was attached to the wall of the pulp canal in several locations ('a' in fig. 122; 'e' in fig. 125). It is unusual to find cementum forming in the pulp tissue of dogs' teeth.

Blood vessels were prominent, appearing dilated and filled in the main with amorphous material (fig. 121). Transitional sites between this intravascular material, which was strongly basophilic in reaction, and blood cells were seen (fig. 123, d). While the distention of the blood vessels may have been due to the Ledermix cement, the intravascular amorphous material, which was noted in untreated control teeth as well, was probably the result of some interaction between the perfusing agent and the blood. This substance, occasionally assumed a segmented appearance (fig. 126). A search of the literature did not reveal a previous description of this phenomenon.

The apical region of the pulp was normal and the periapex undisturbed (fig. 127).

Fig.117

Inst-ray
L.P.
H.P. Combination

Fig.118 x20

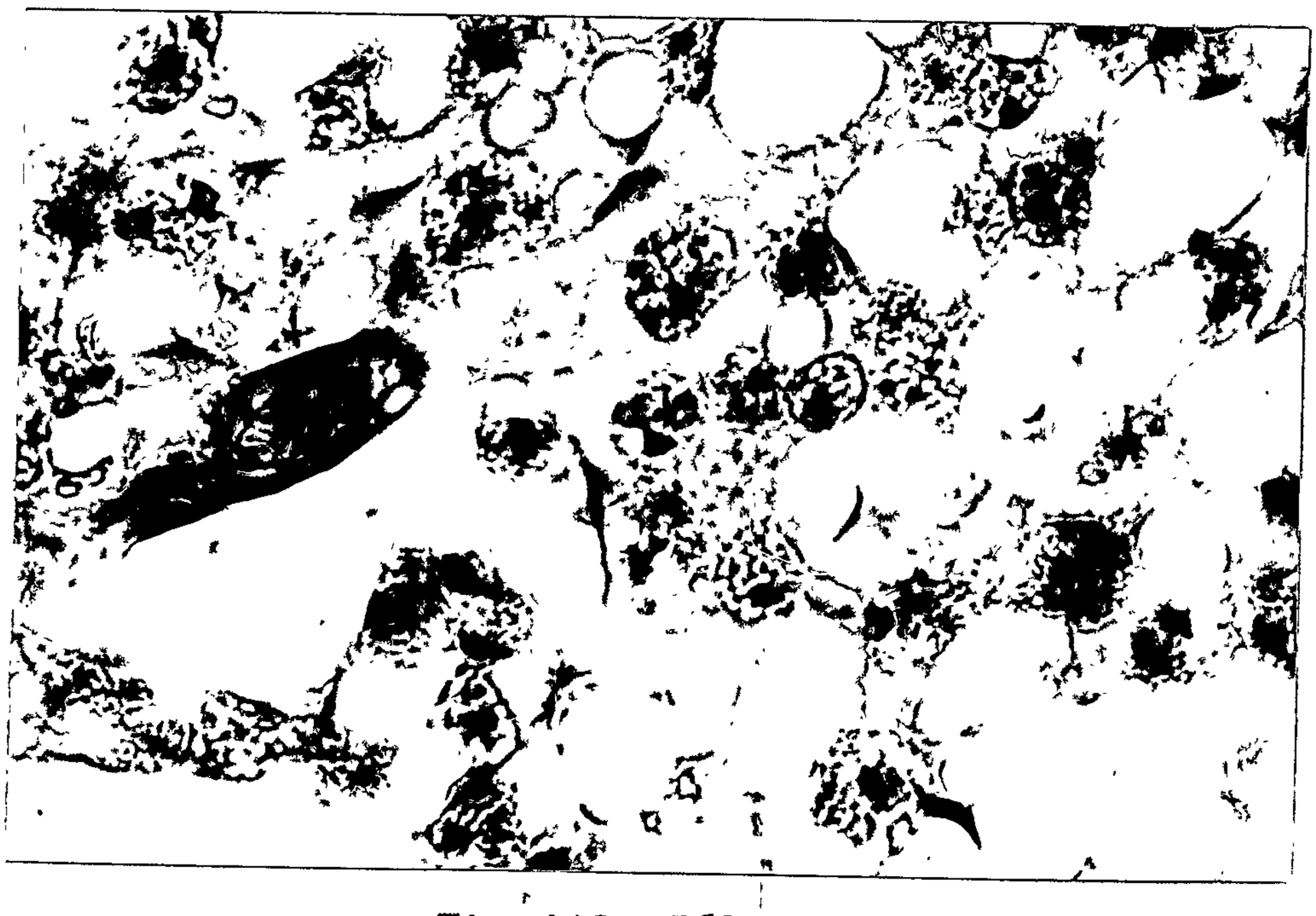


Fig.119 x560

Fig.120 x30

Fig.121 x30

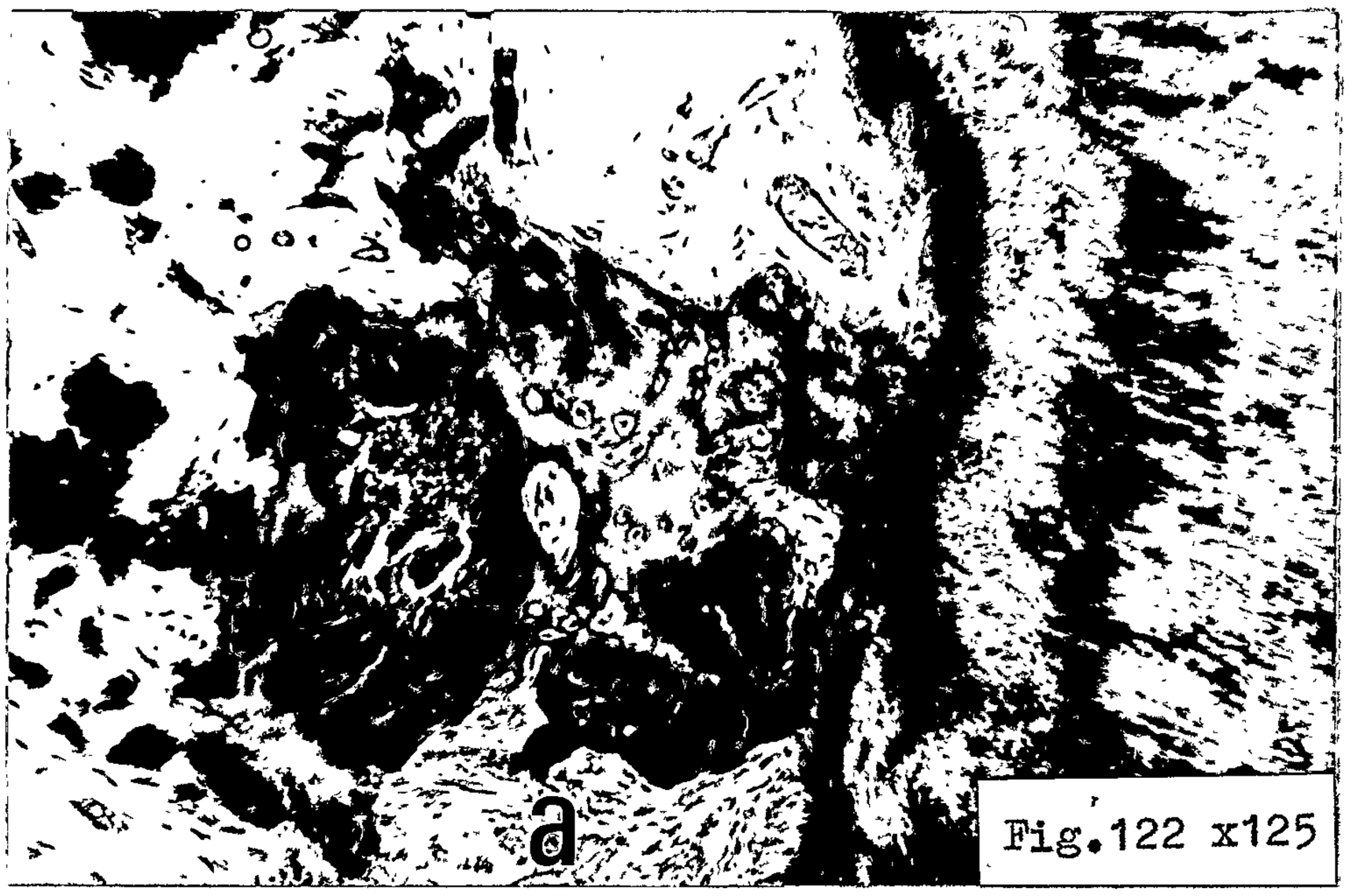


Fig.122 x125

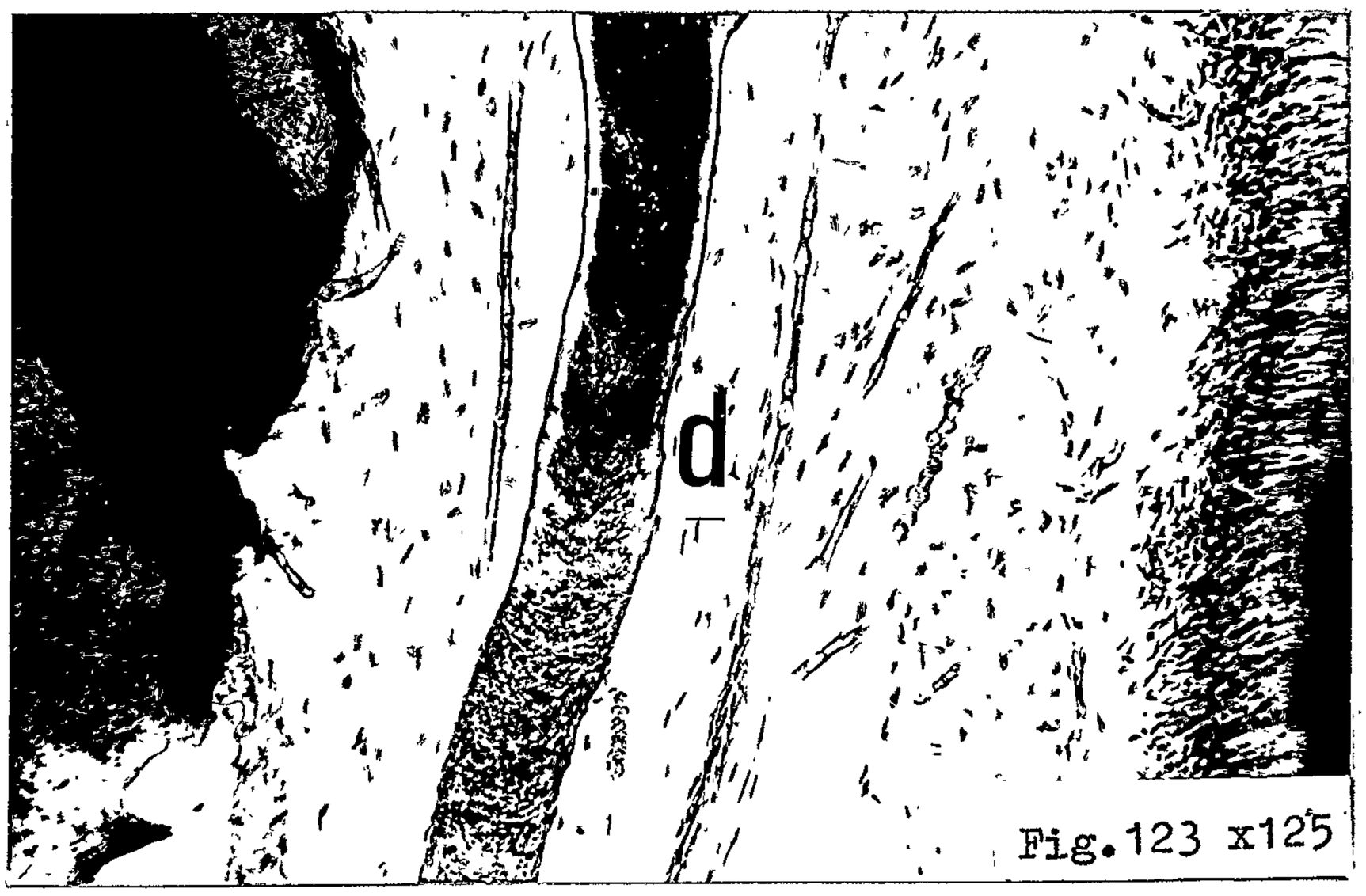


Fig.123 x125

Fig.124 x30

Fig.125 x125

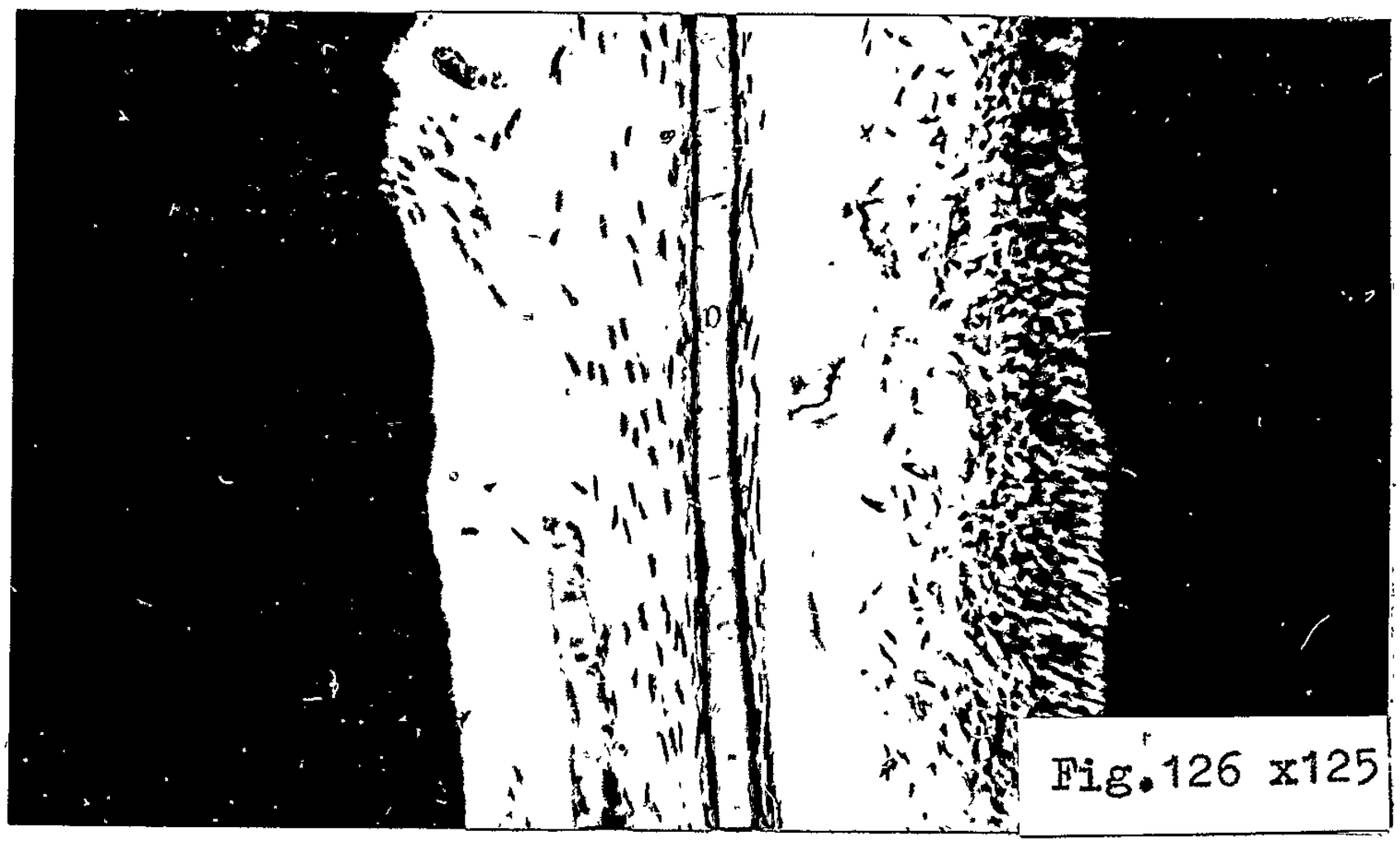


Fig.126 x125

Fig.127 x18

Experiment 25. Figs. 128 - 130, page 131.

Ledermix cement had been applied to the exposed mesial and distal pulp cornua of the lower right third premolar for a period of 2 months (a, fig. 128).

In this case, some disorganisation of the pulp tissue was observed and the possibility of artifact due to inadequate fixation must be considered. In the mesial canal the pulp appeared fibrosed and had become detached from the canal wall, and the peripheral odontoblasts were reduced in size and number (figs. 129,130). Distally, dilated blood vessels and areas of haemorrhage were prominent features but photomicrographs of this canal have not been included.

The periapex retained its normal architecture (fig. 129).

Experiment 26. Figs. 131 - 134, page 132.

Ledermix cement was inserted into a cavity exposing the distal part of the coronal pulp in a lower left third premolar and left in place for 6 months.

Degenerative changes noted in the pulp may again be the result of inadequate fixation, but some features were possibly attributable to the capping procedure. Beneath the cement, the pulp tissue appeared oedematous and reticulated and the stroma presented a strong basophilic tendency. The blood vessels were generally dilated and filled with basophilic material (fig. 132). The condensation and detachment of the pulp tissue from the canal walls in some locations (fig. 131) and the reduction and disorganisation of the odontoblast layer throughout may be artifact however.

An unusual formation was observed in the apical region of the distal root. Here there had been an ingrowth of very cellular hard tissue resembling cementum into the canal lumen. This aberrant cementum was fused to the apical cementum and projected from the commencement of the apical ramifications (figs. 133, 134). This phenomenon was not observed in any other root canal.

Fig.128

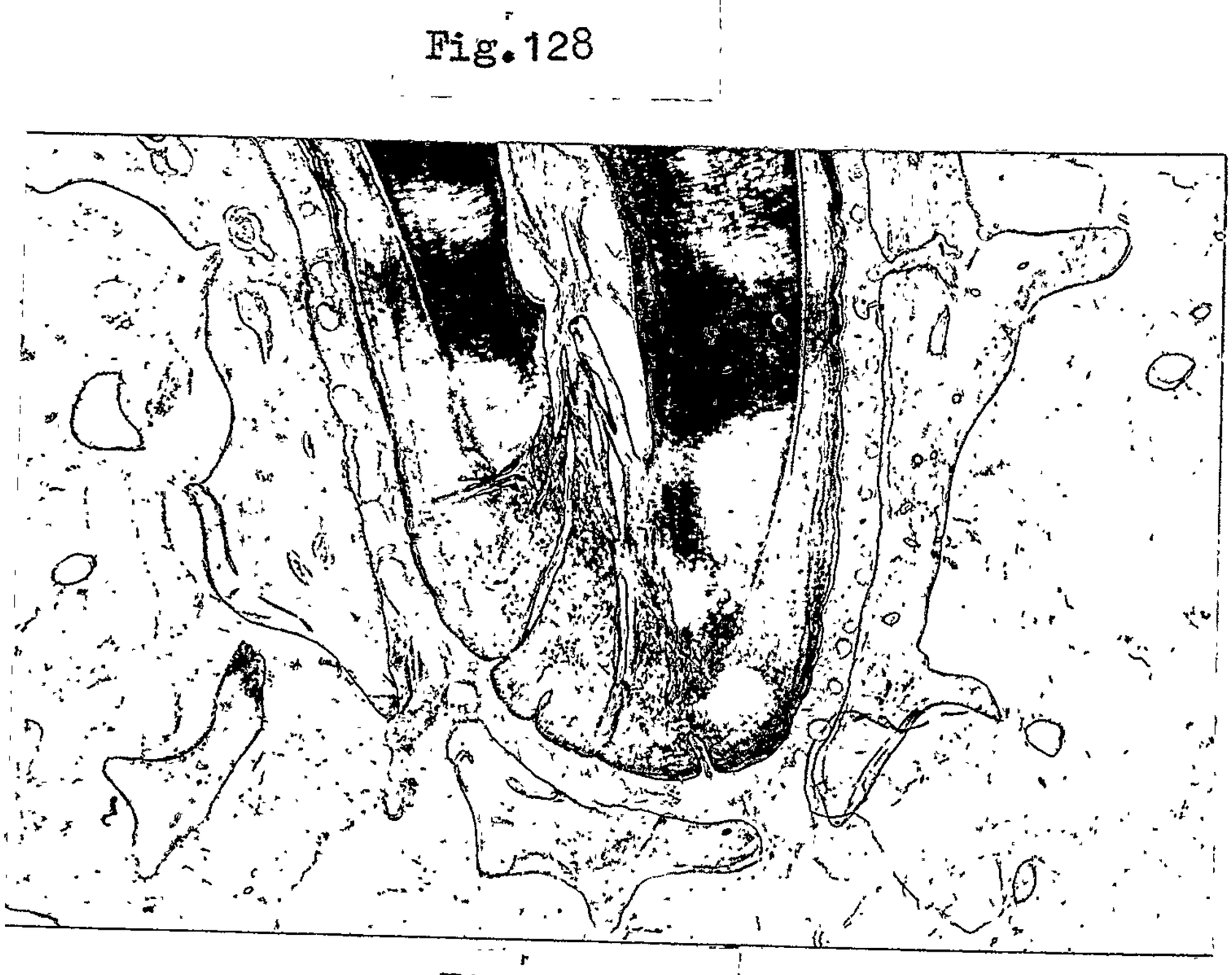


Fig.129 x17

Fig.130 x125

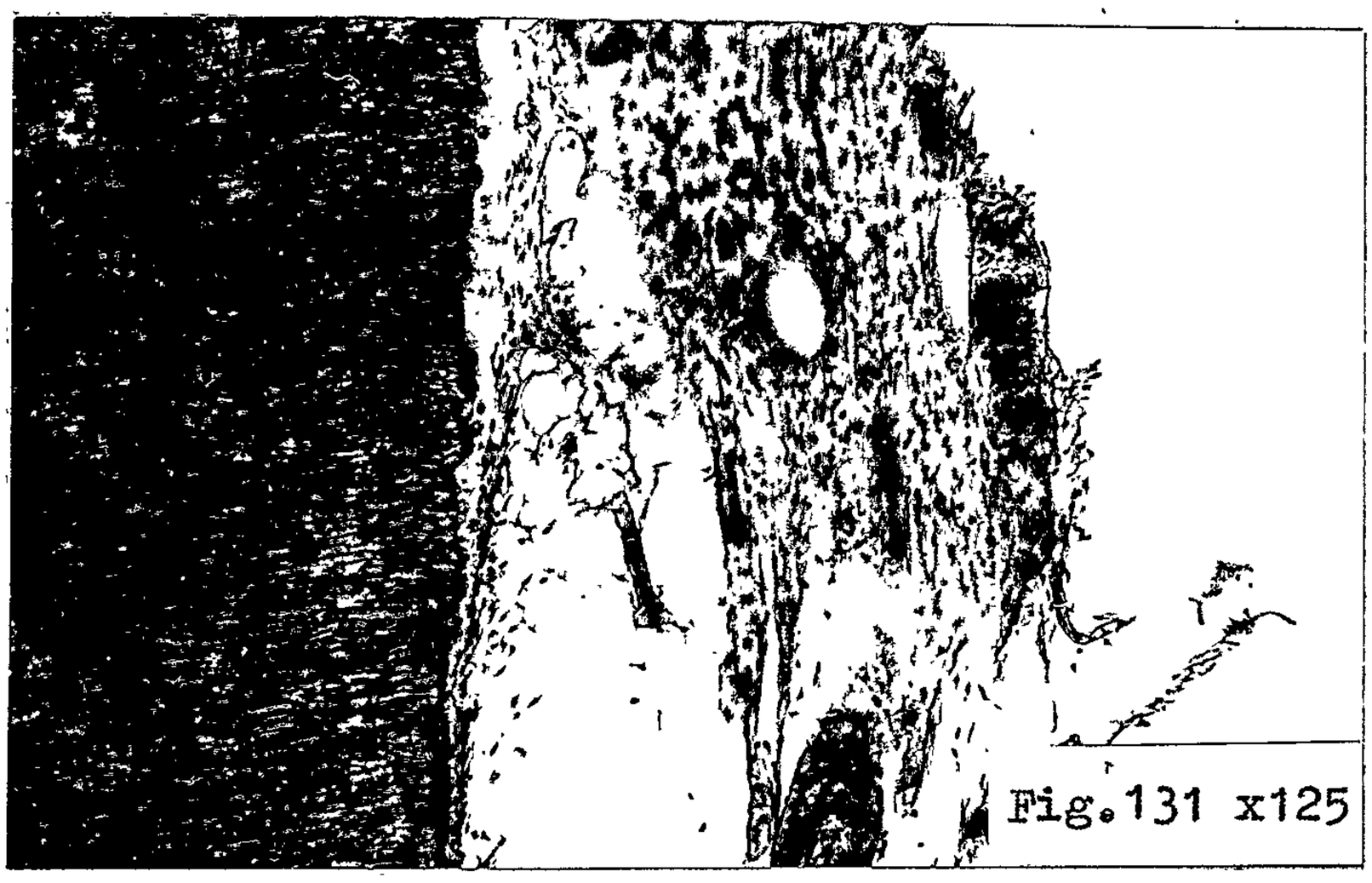


Fig.131 x125

Fig.132 x125

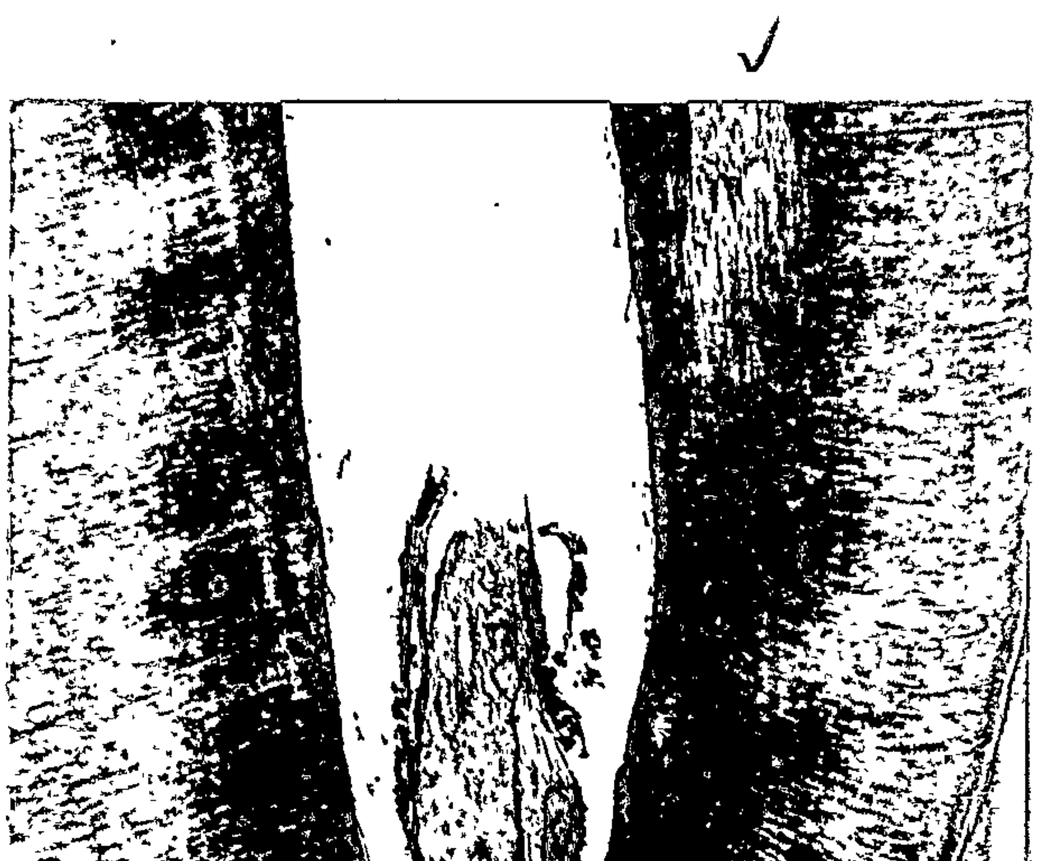


Fig.133 x30

Fig.134 x125

Experiment 27. Figs. 135 - 137, page 134.

Ledermix cement had been applied with moderate pressure to a pulp exposure in the distal part of the crown of a lower right premolar and allowed to remain in place for 3 months. Sections showed that a considerable quantity of the material had been forced into the pulp tissue but there was no evidence of an inflammatory response (fig. 135). Odontoblasts and the predentine layer were absent from the coronal pulp and had disappeared from the distal canal deep to the cement. In the mesial root canal however, both odontoblasts and predentine were intact and at the site of their commencement (a, fig. 135) there was marked basophilia. Deeper sections of the pulp showed vacuoles appearing beneath the cement in the coronal part of the pulp (fig. 136). Since there was an absence of pulp disorganisation and tissue compression around these spaces, the possibility that the vacuolisation was caused by the capping procedure cannot be entirely dismissed. The remainder of the pulp appeared vital and a photomicrograph of the periapical area showed an intact lamina dura (fig. 137).

Experiment 28. Fig. 138, page 135.

Application of pressure to the Ledermix cement had forced the material into the pulp through a distal exposure in a lower right second premolar. The odontoblast layer was not apparent in the distal canal but was preserved in the mesial canal. Dentine chips lay free in the pulp causing no reaction and the pulp appeared normal throughout. Coronal to the cement an area of secondary dentine had been elaborated. The duration of the experiment was 8 months.

Experiment 29. Figs. 139, 140, page 135.

Ledermix cement was applied to an exposure of the distal pulp cornu in the lower right second premolar (fig. 139) and the experiment was allowed to continue for 3 months. At the exposure site dentine chips and cement were seen in the pulp tissue but there was no reaction and the pulp appeared normal. Dilated blood vessels were prominent beneath the exposure.

Fig.135 x10

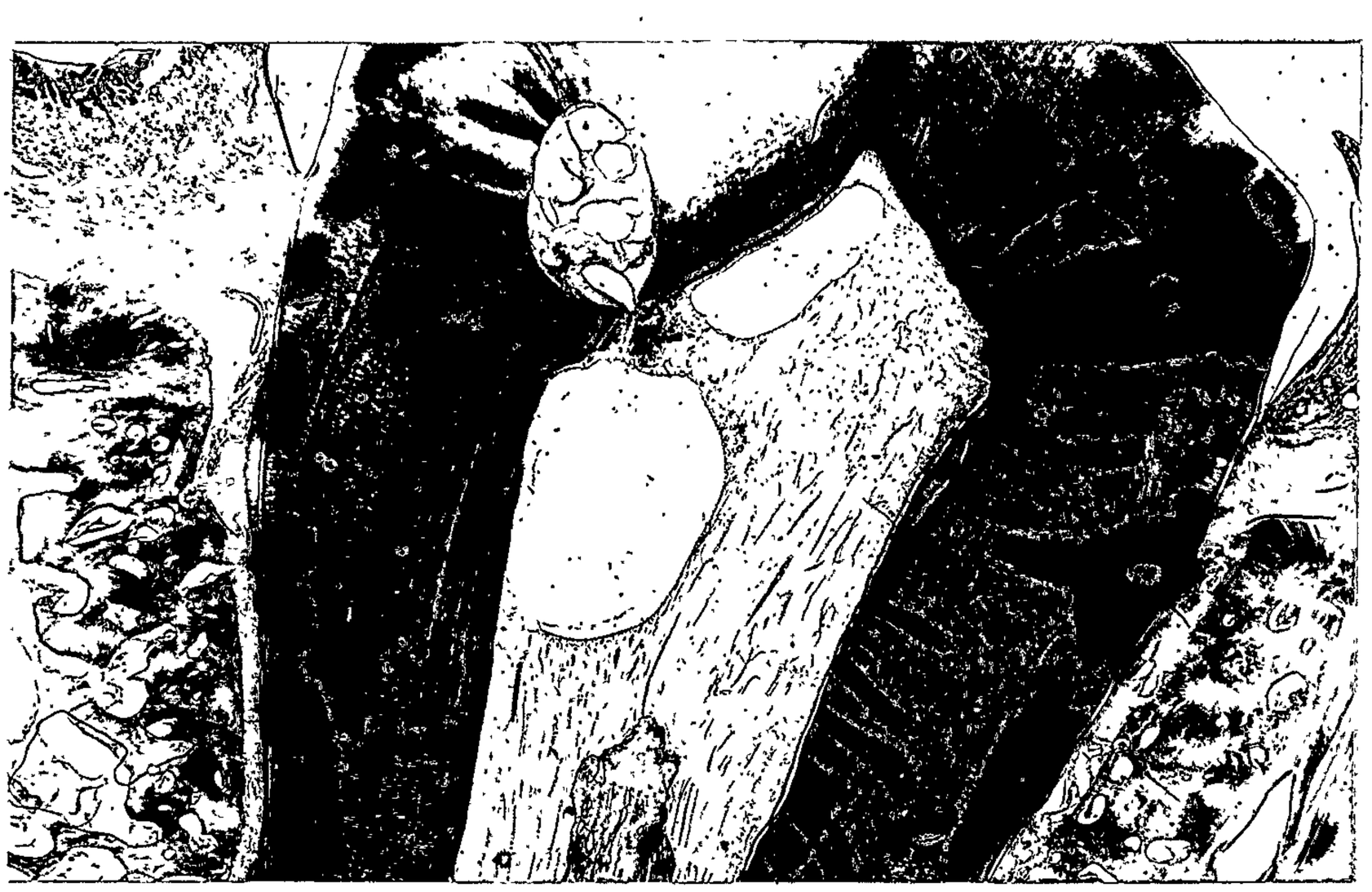


Fig.136 x9

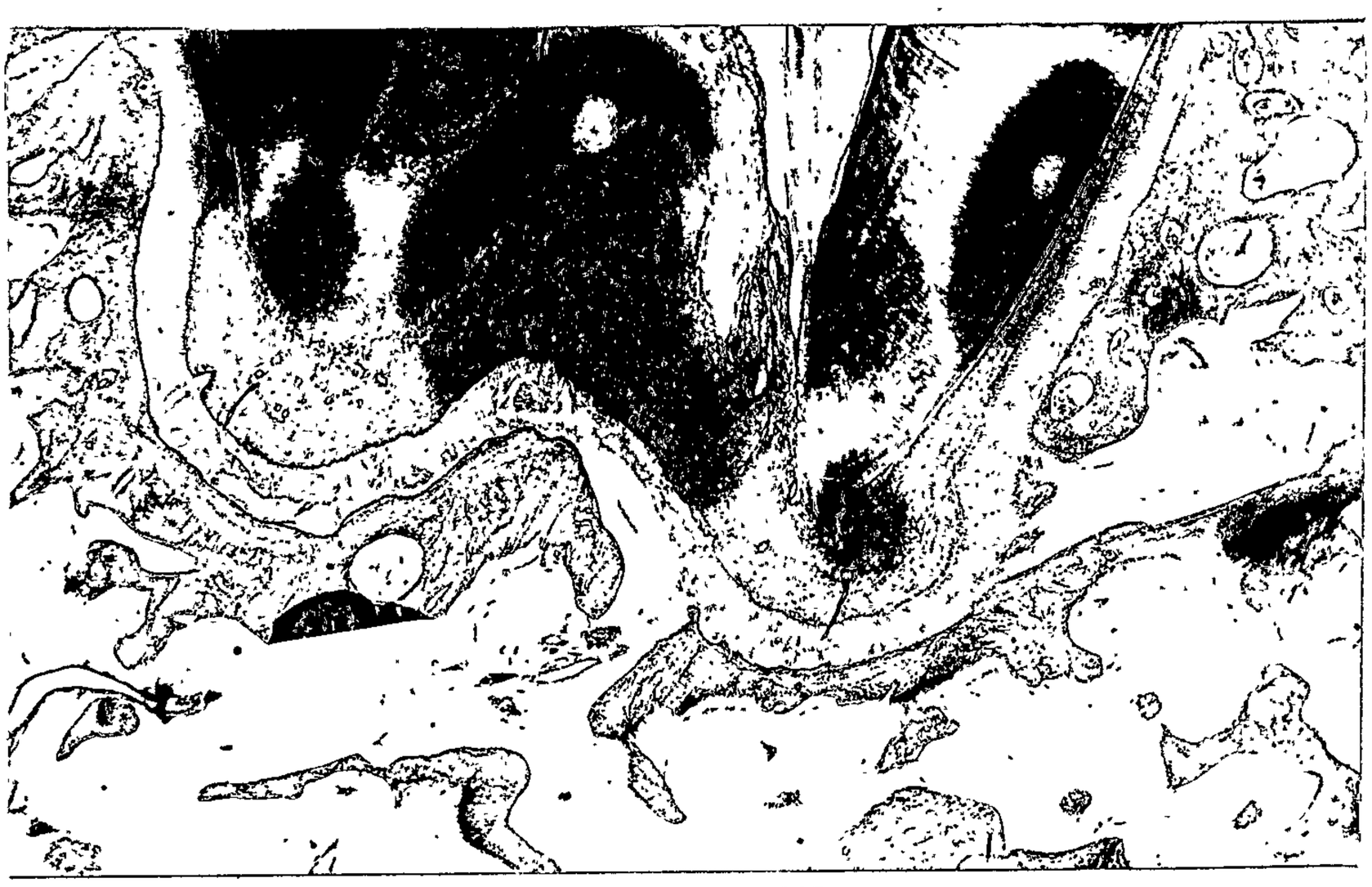


Fig.137 x12

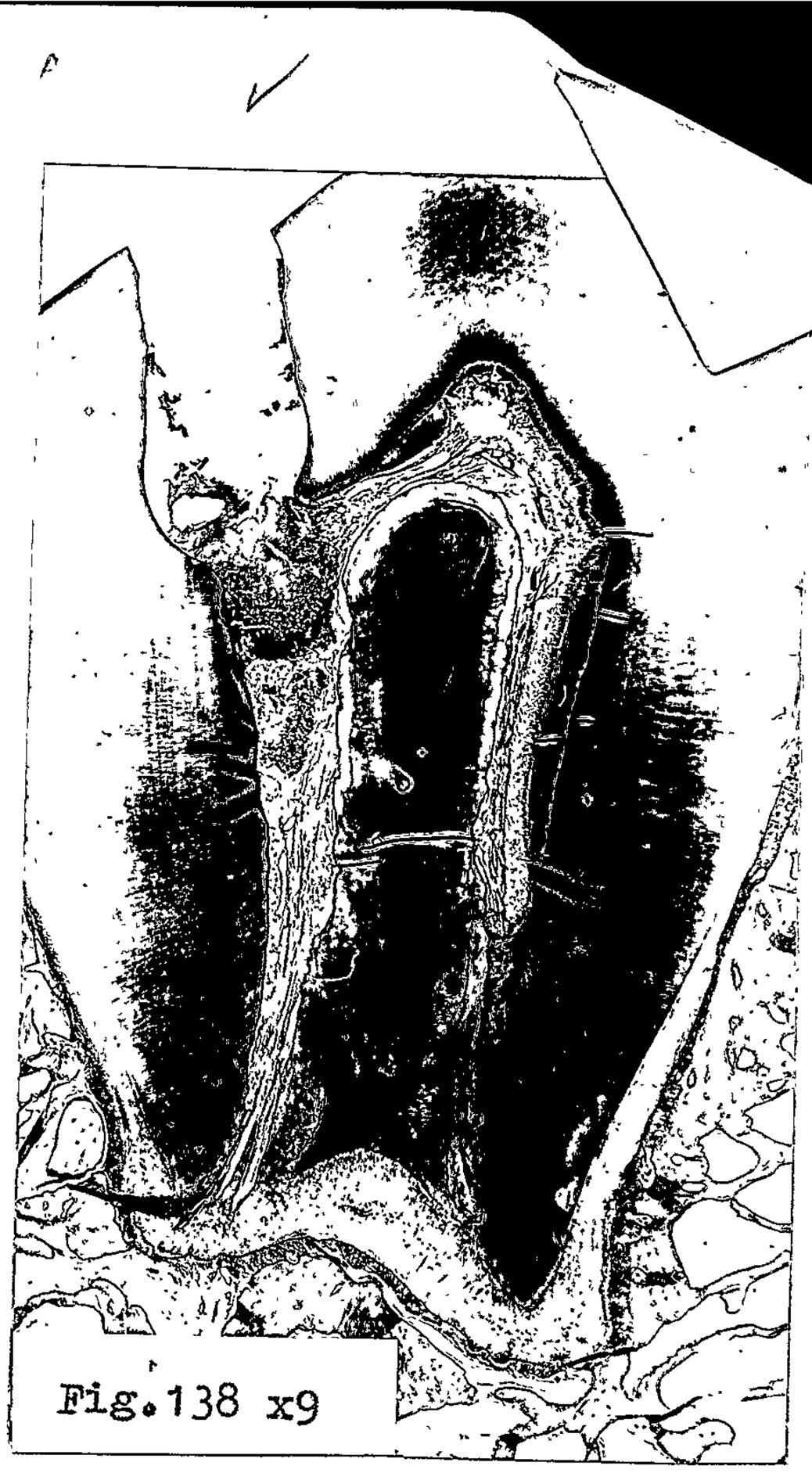


Fig.138 x9

Fig.139

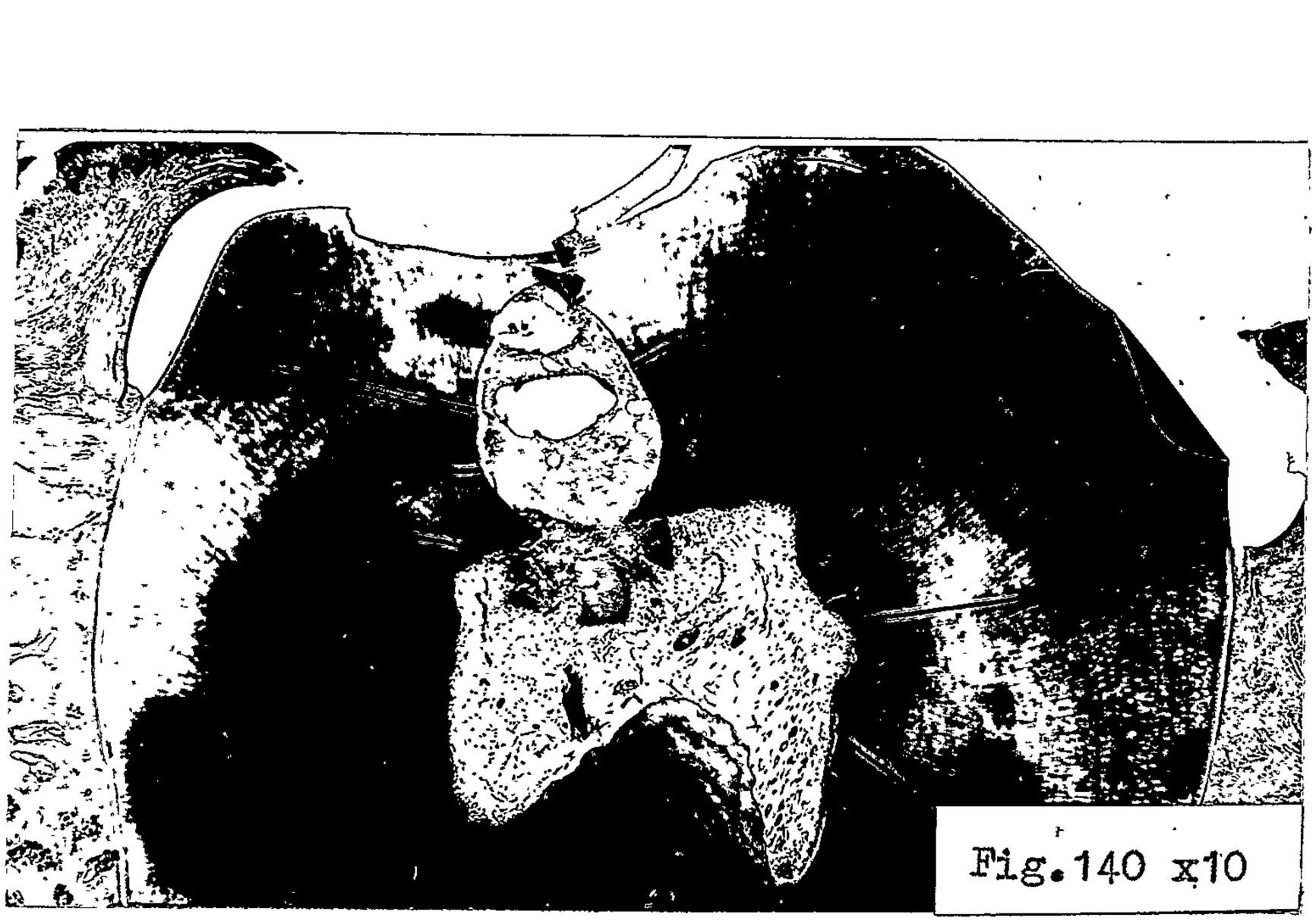


Fig.140 x10

Experiment 30. Figs. 141 - 143, page 137.

Ledermix cement had been forced into the distal pulp horn of the lower left 4th premolar (fig. 141) and was left in place for 3 months. Some loss of pulp structure occurred coronally with the formation of vacuoles but these may be artifact. The odontoblast layer was retained, and this observation, together with the presence of dilated blood vessels, suggested that the pulp had been vital at the time of sacrifice (fig. 142). Dentine chips forced into the coronal pulp were encased in newly formed hard tissue, and this mass had become attached to the wall of the pulp chamber in one location (fig. 143).

Experiment 31. Fig. 144, page 138.

Ledermix cement, which had been flowed over the exposure site in the lower premolar without pressure, caused no reaction in the pulp after 3 months. Dentine chips lay free in the pulp tissue and some blood vessels were dilated. As seen in other instances, the odontoblast layer had disappeared from the canal wall deep to the exposure but was present elsewhere.

Experiment 32. Figs. 145, 146, page 138.

Ledermix cement had been inserted deeply into the pulp exposure distally and allowed to remain for four months.

The odontoblast layer had been reduced in all parts of the pulp except the mesial canal and the coronal pulp was loosely fibrillar with few formed elements. Along with the disappearance of the odontoblasts there was a reduction in the predentine layer (fig.145). A small area of resorption of dentine was noted adjacent to the cement (r). A high power photomicrograph (r, fig. 146) showed small cells in the area but it is unlikely that they were responsible for the resorption.

Experiment 33. Fig. 147, page 138.

After 8 months the pulp tissue deep to the layer of Ledermix cement appeared normal apart from the presence of some dilated blood vessels.

Fig.141

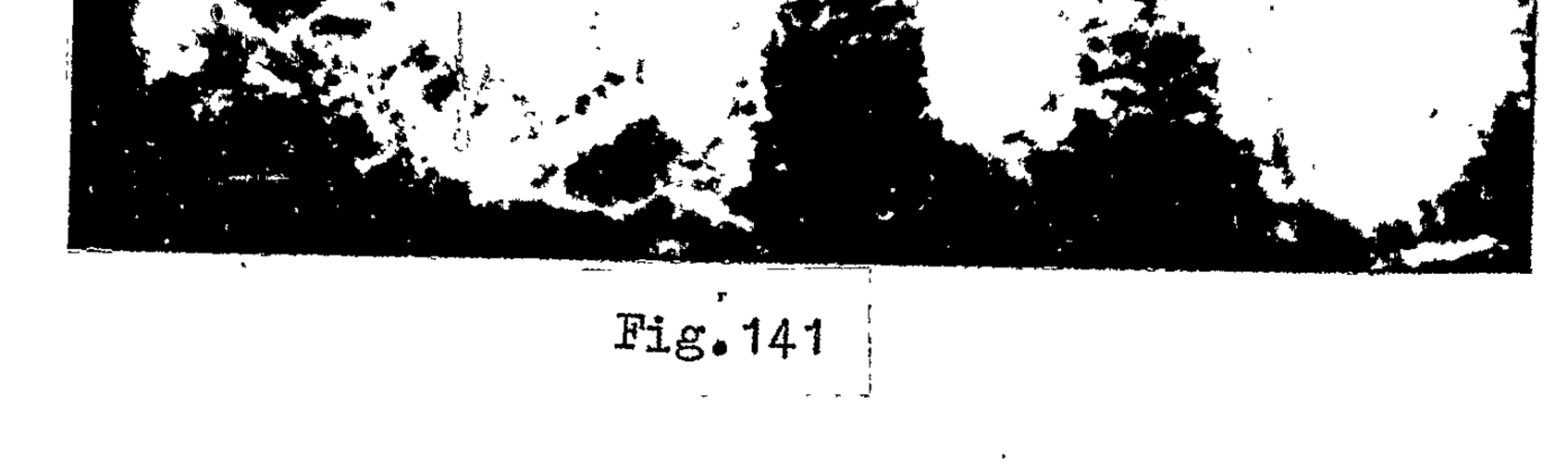


Fig.142 x11

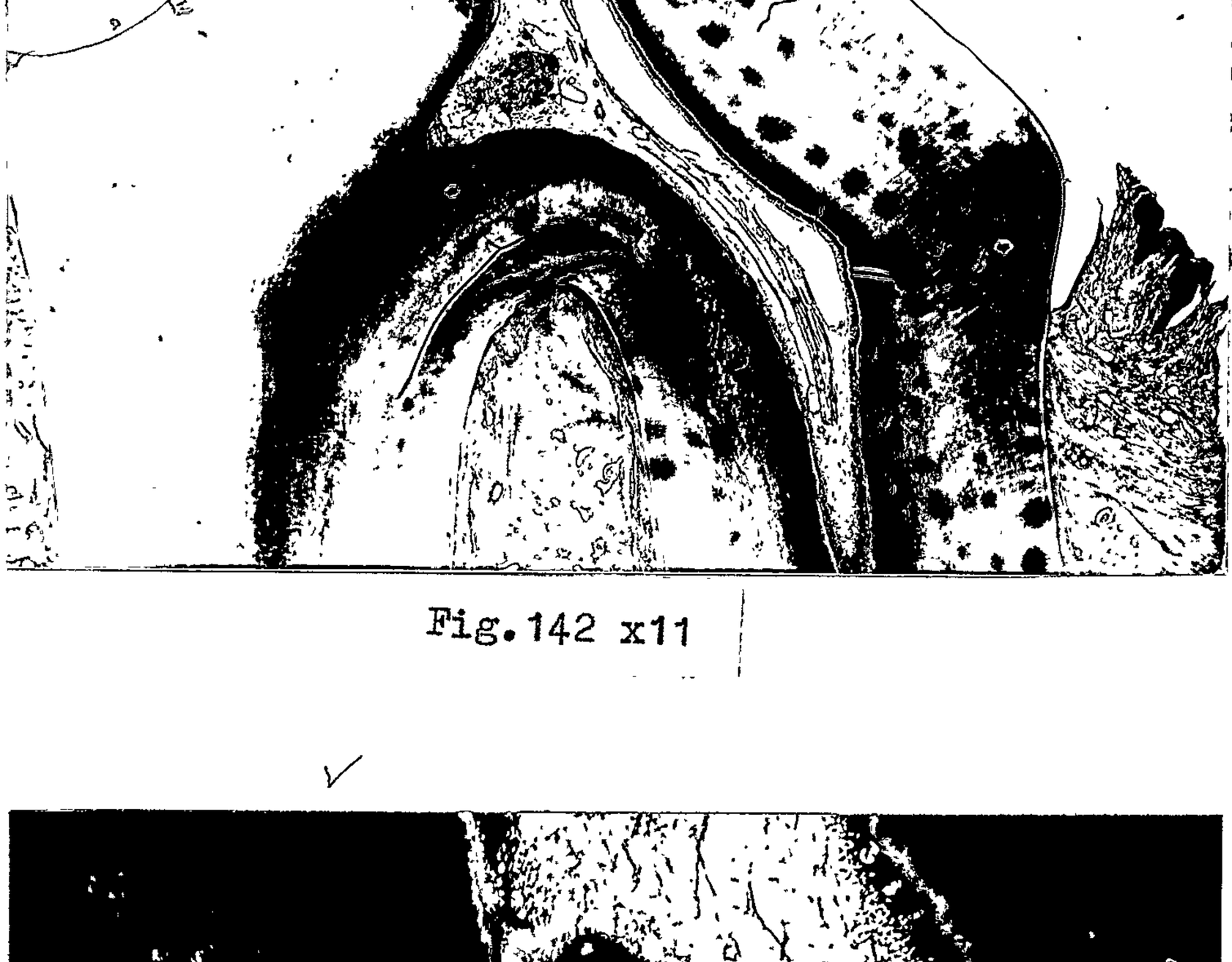


Fig.143 x44

Fig.144 x15

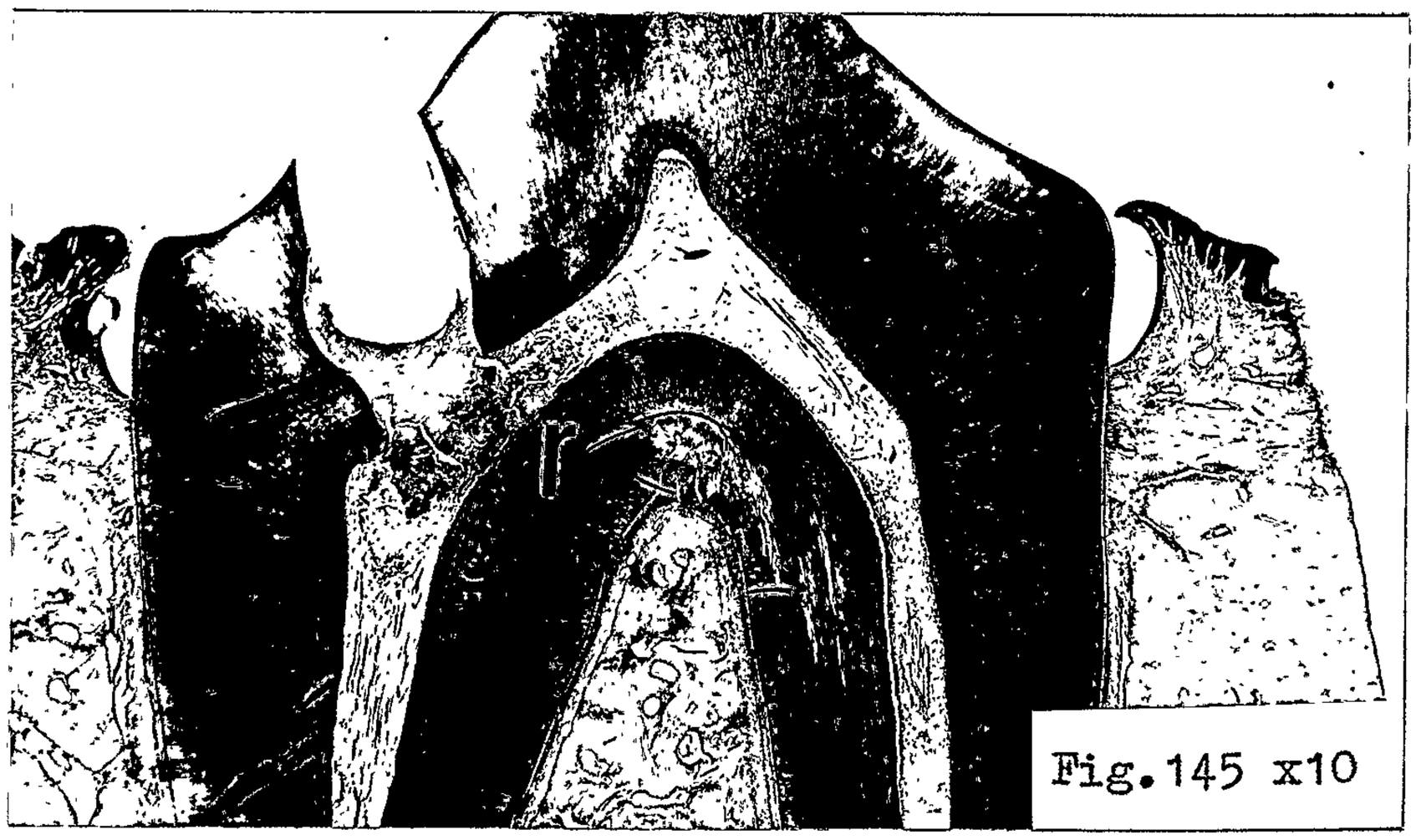


Fig.145 x10

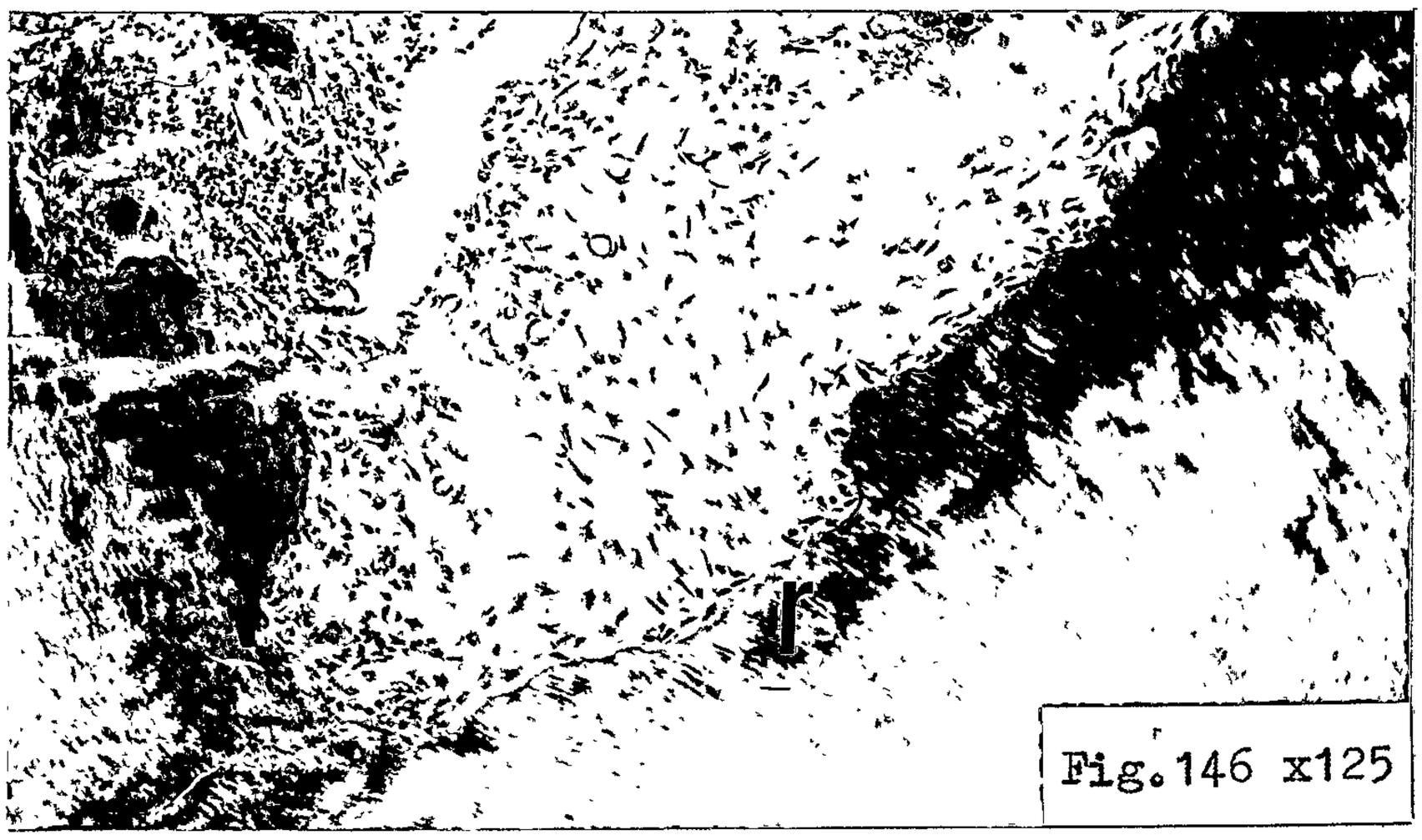


Fig.146 x125

Fig.147 x26

Experiment 34 A,B. Figs. 148 - 1518, page 140, 141.

Ledermix paste was applied to a distal exposure in a lower right second premolar, and Cortril, which contains hydrocortisone in a water miscible base, was inserted into a cavity exposing the mesial pulp.

After a period of two months, the pulp tissue beneath the Ledermix paste appeared to have undergone a degeneration to a filamentous structureless remnant without any cellular elements (Figs. 148, 150). Vital tissue persisted in the apical ramifications and the periapical region appeared normal (Figs. 151, 148). The junction between the degenerate tissue in the canal and the vital tissue at the commencement of the apical ramifications was marked by a transitional zone of cells whose nuclei were pyknotic (Fig. 149).

Mesially, Cortril had caused complete pulp destruction and an acute apical reaction had supervened (Fig. 151 A).

Application of Cortril to exposed mesial and distal pulps in another tooth, a lower left second premolar, also caused acute apical abscess formation (Fig. 151 B).

Experiment 35. Figs. 152 - 154, page 142.

Following pulpotomy in the mesial and distal canals of the lower premolar shown in Figs. 152 to 154, Ledermix paste was applied for one month. The photomicrographs showed degeneration of the pulp tissue in each canal. In the distal canal, the pulp had been virtually completely lost (Fig. 153). Dentine chips lay free in the residual tissue and had caused no reaction. Fig. 154 shows that the periapical tissues remained normal and vital tissue filled the ramifications.

Degeneration of the pulp such as seen in this experiment and others previously described must be interpreted cautiously in view of the possibility of artifact due to inadequate fixation. This possibility may be considered remote, however, in view of the lengthy perfusion of the animal with formol saline followed by immediate dissection of the jaws and immersion in fixative for a period of at least two weeks. Untreated teeth from each animal were examined and normal pulp histology was usually demonstrated.

Fig.148 x20

Fig.149 x125

Fig.150 x125

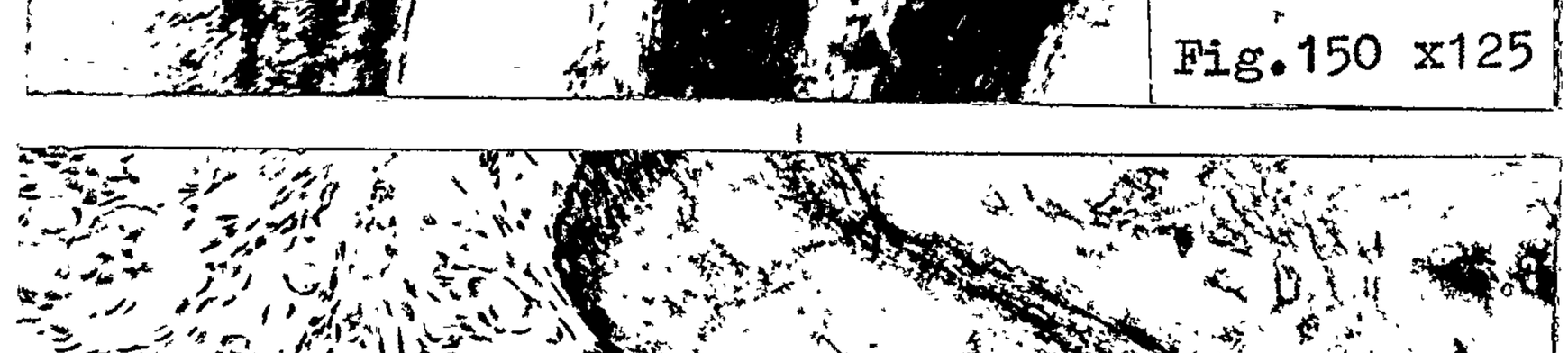


Fig.151 x125

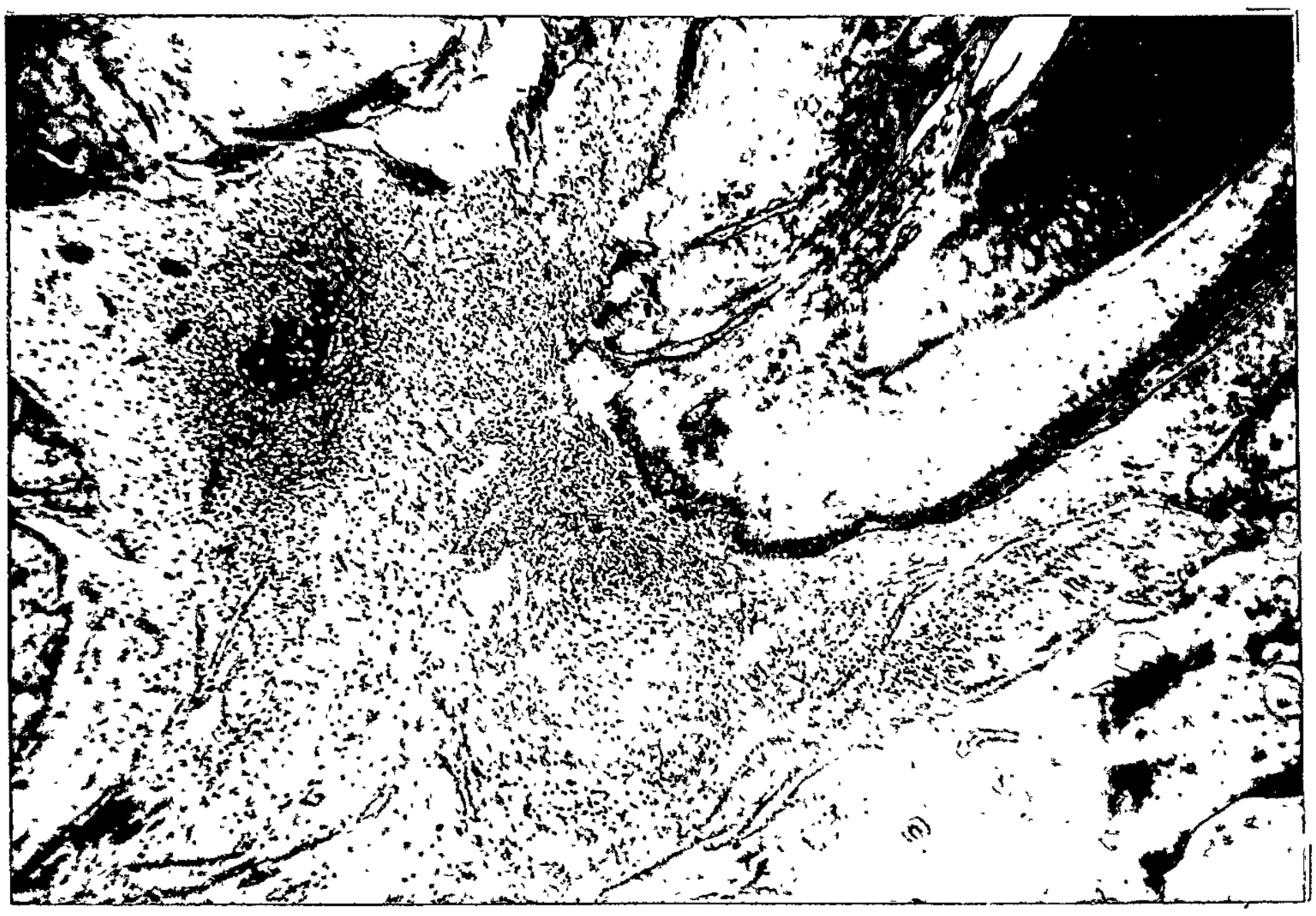


Fig 151a x32

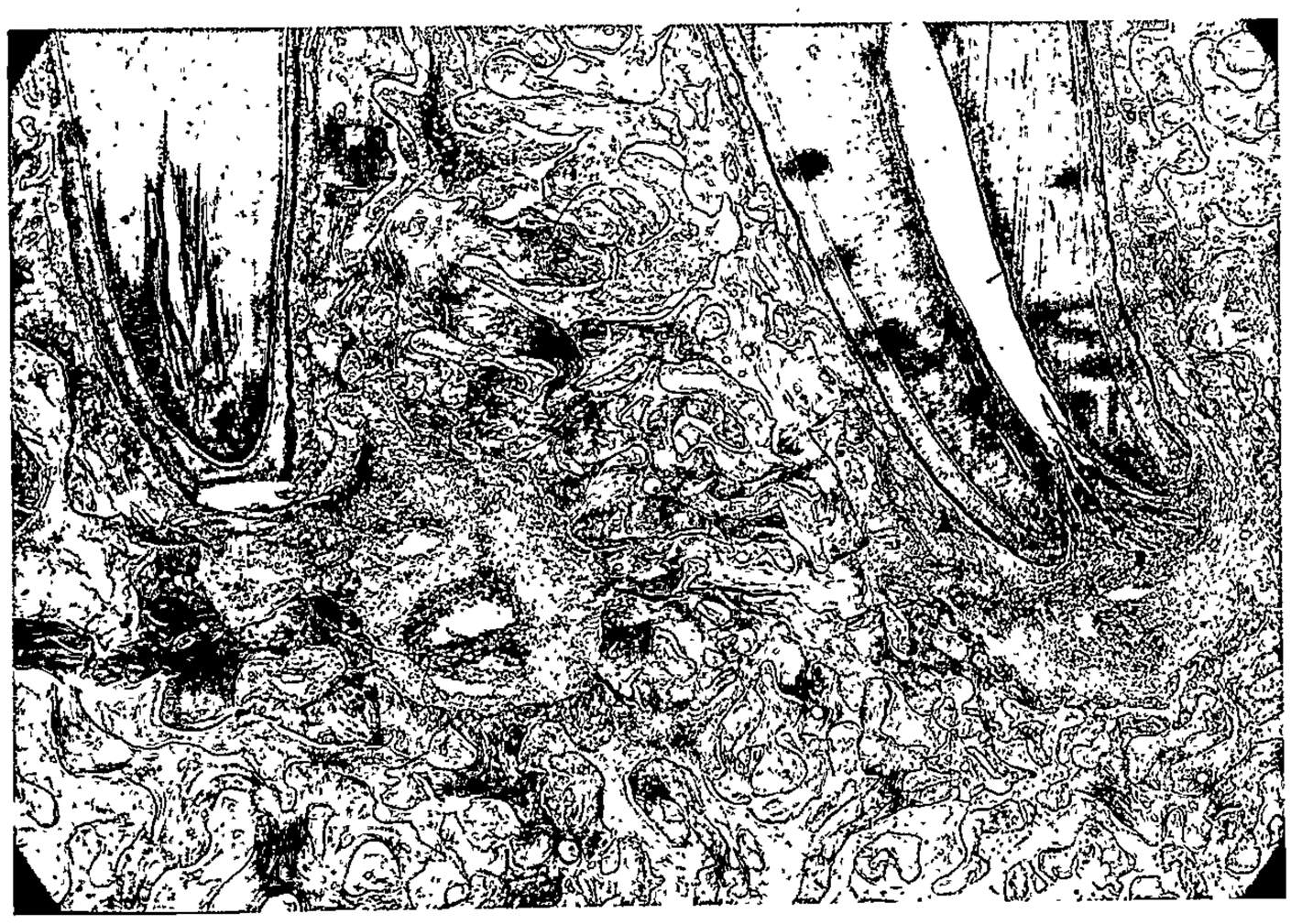


Fig 151b x10

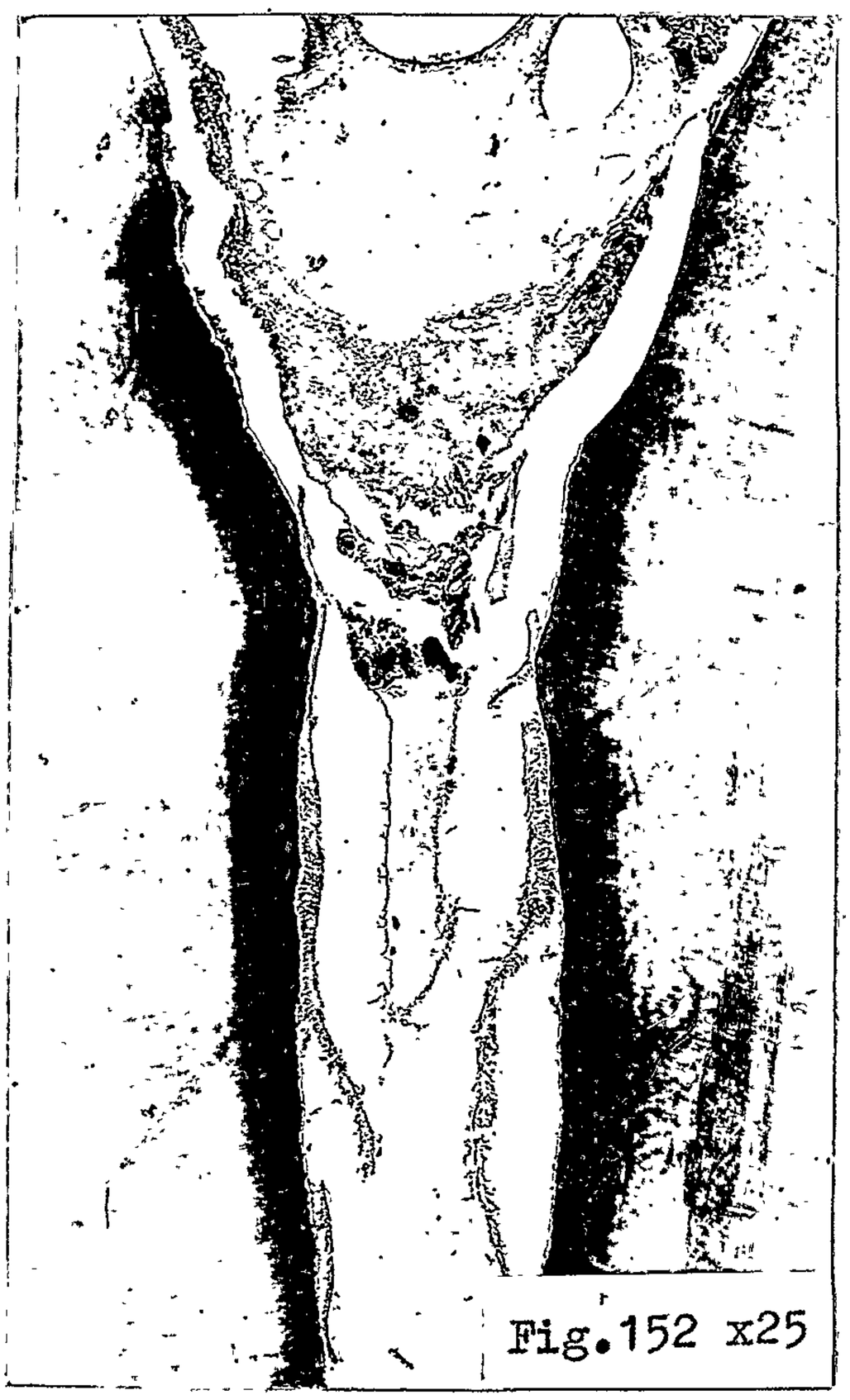


Fig.152 x25

Fig.153 x25

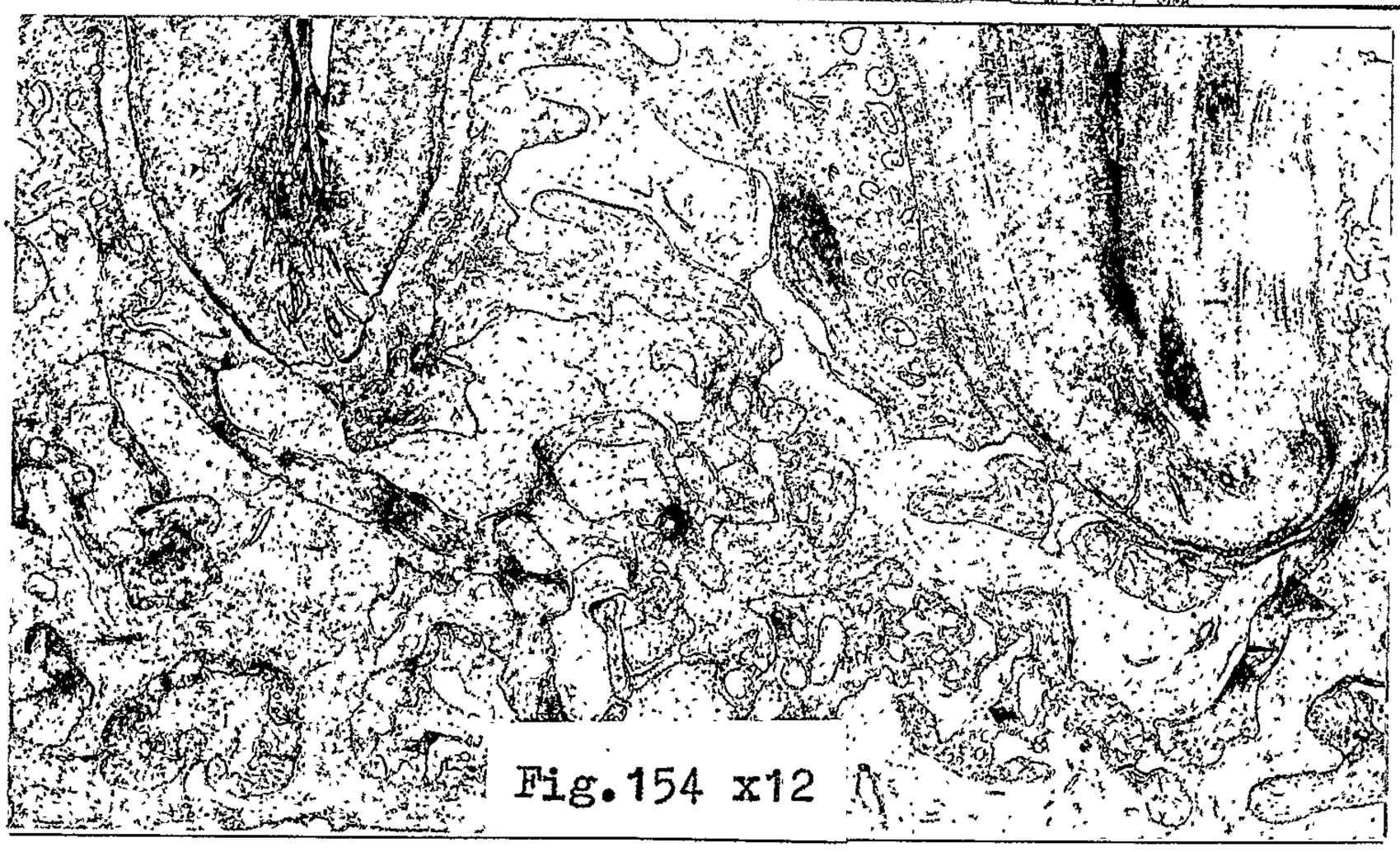


Fig.154 x12

Fig.155 x30

Fig.156 x30

Experiment 36. Figs. 155, 156, page 142.

Ledermix paste on cotton wool had been placed in contact with the pulp tissue as shown in Figs. 155 and 156. After 6 months, this pulp appeared to have retained its vitality and there was only slight reduction of the odontoblast layer near the exposure. Dentine chips which had been forced into the pulp were encased in newly formed hard tissue (c) and this was seen to be attached to the canal wall at one site (c, fig. 156).

Experiment 37. Figs. 157 - 162, page 144, 145.

Streptococcus viridans had been used to develop areas of reaction around the apices of the lower right 3rd and 4th premolars (fig. 157). It was decided to use the lower right 4th premolar to test the efficiency of Ledermix paste in eradicating bacteria and to see whether the paste had any inhibitory effect on osteogenesis and healing of the apical region.

The two canals of the lower right 4th premolar were reamed and filed to the cemento-dentinal junction and Ledermix paste was inserted for 2 weeks. Following this treatment, the paste was irrigated out of the canals and root filling with gutta percha points and root canal sealer was completed. The lower right 3rd premolar was reamed and filed to the start of the apical ramifications and after dressing the canals for 5 minutes with camphorated monochlorphenol, root filling was carried out in a similar manner to the 4th premolar (fig. 158). The animal was sacrificed 4 months later.

Histologic sections showed that healing had occurred almost completely in each case, although small foci of inflammatory cells persisted around some canal openings (figs. 159, 160). Restoration of bone had occurred in each apical region. (High power inset Fig. 160 shows bone trabeculae forming).

The staining of bacteria with Gram was unsuccessful in the lower right third premolar, but in the distal apex of the 4th premolar, pockets of bacteria persisted in the cemental lacunae and in the canal ramifications at the apex (e, figs. 161, 162).

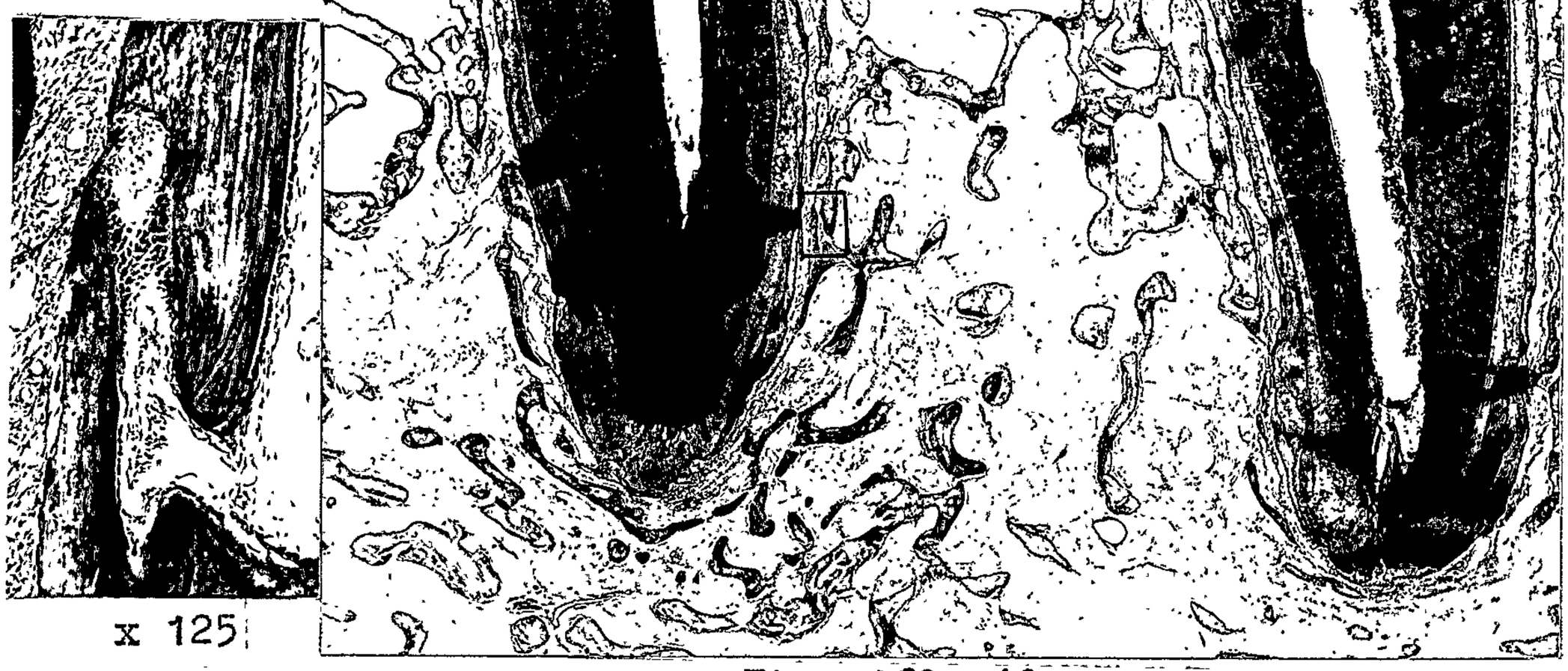
In this experiment, therefore, Ledermix paste was not effective in completely eradicating bacteria from the root apex, but the material did not appear to hinder the healing processes in the periapical area.

d m

Fig.157

Fig.158

Fig.159 x11
3rd.premolar



x 125

Fig. 160 x10
4th. premolar

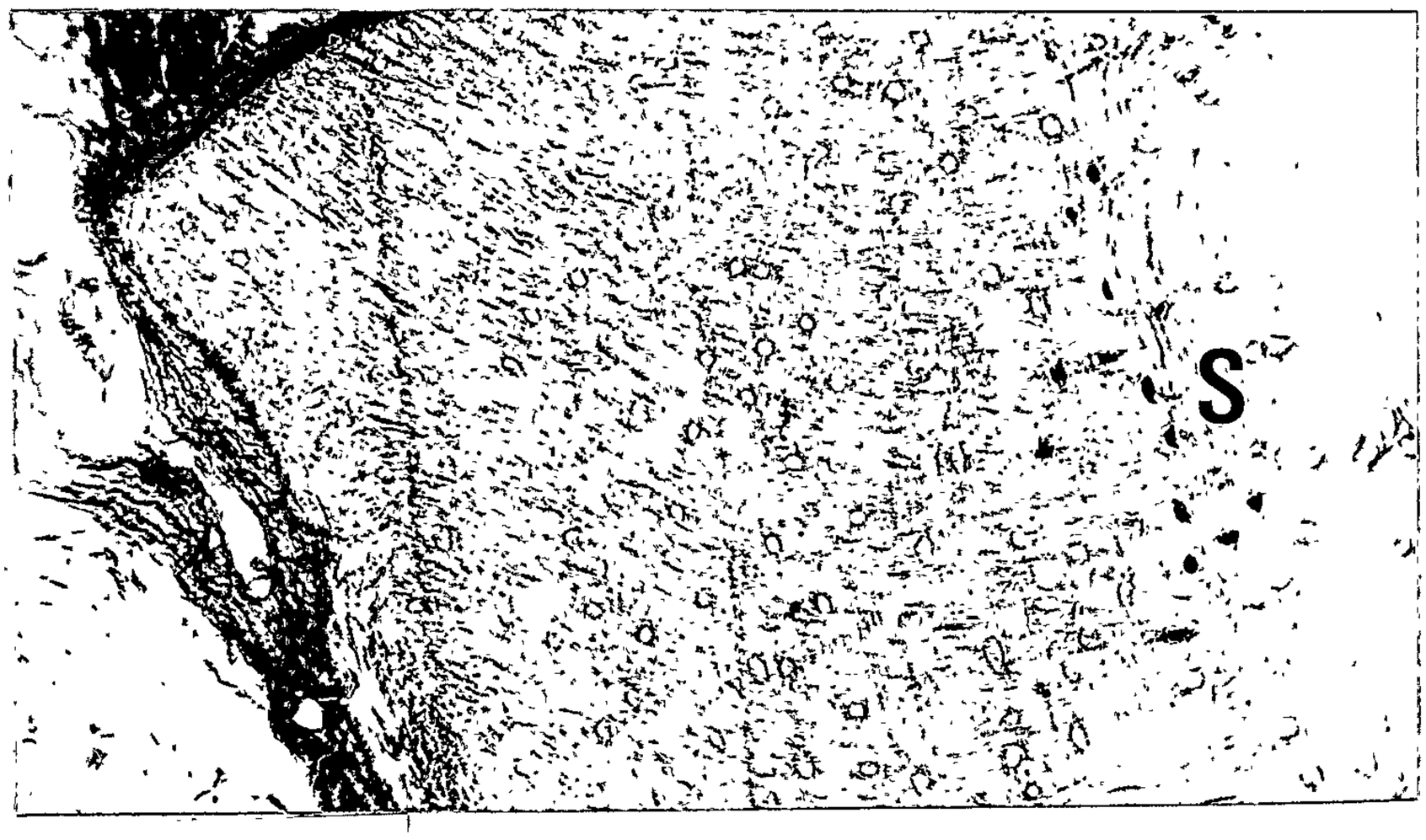


Fig.161 x125
Gram

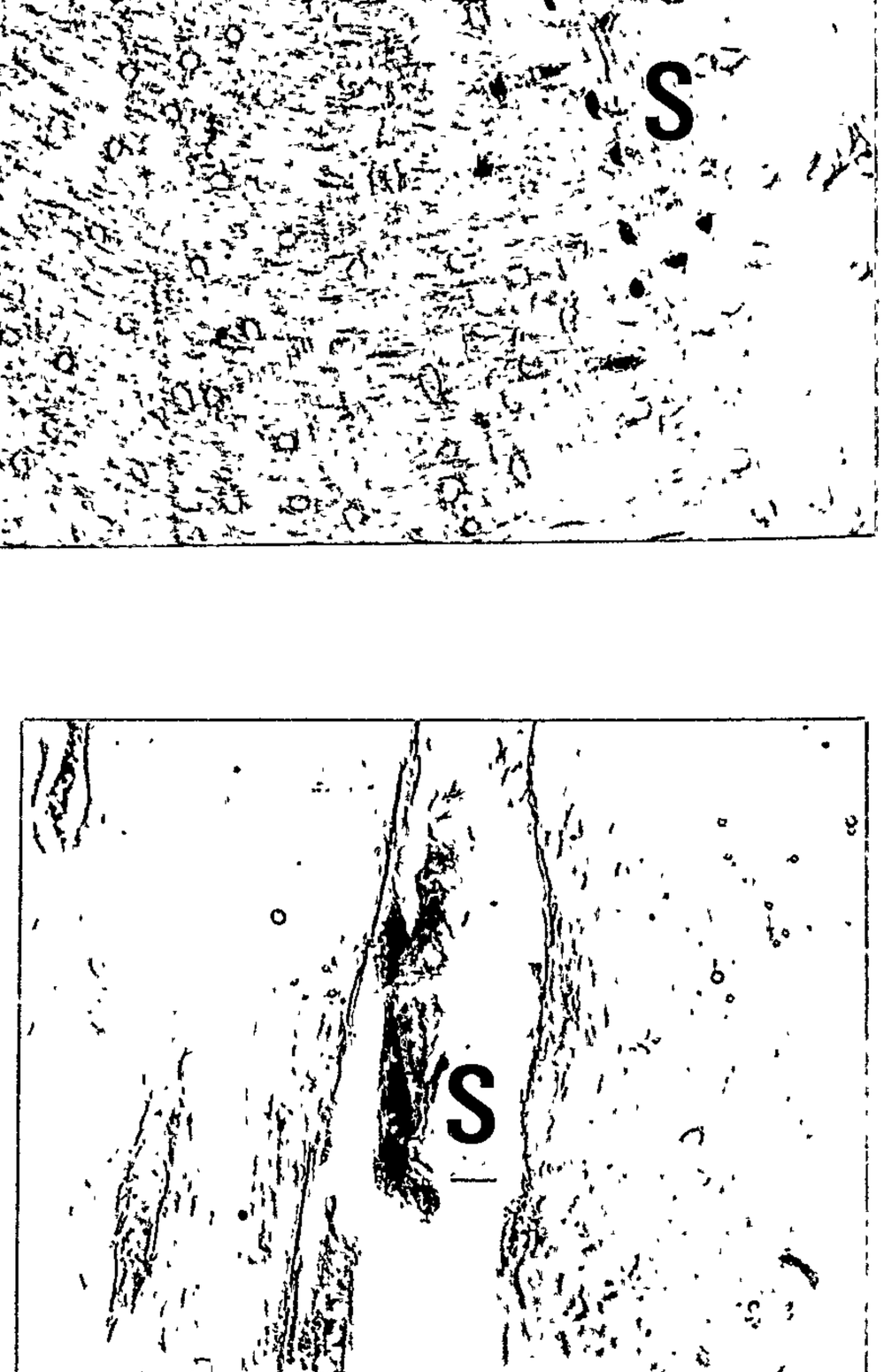


Fig.162 x125
Gram

Experiment 38. Figs. 163 - 165, page 147.

Streptococcus viridans had produced areas around the root apices of the lower right 4th premolar after 4 months (fig. 163). The canals were reamed and filed to the start of the ramifications and Ledermix paste plus a gutta percha point inserted in the mesial root, and the distal root was filled with root canal sealer and gutta percha points.

At the time of sacrifice 3 months later, the areas were still apparent on the radiograph, although it is possible that some reduction in size had occurred (fig. 164).

Sections of the mesial root apex showed that the large area had been filled in with connective tissue, and although little bone had yet formed, some new trabeculae were apparent (fig. 165 + HP). Some residual inflammation persisted, but Gram stain did not reveal bacteria in either root apex.

In this instance, Ledermix paste did not induce complete subsidence of periapical inflammation even though it had been sealed in the canal for a prolonged period.

Experiment 39. Figs. 166 - 169, page 148.

Areas had developed around the apices of the lower left 4th premolar (e, fig. 166) following inoculation of the root canals with streptococcus viridans. Both apices were perforated and Ledermix paste was inserted ~~inserted~~ into the canals together with a gutta percha point. The animal was sacrificed two months later.

The distal apex was typical of the two. The area of reaction had decreased and loose connective tissue surrounded the extruded gutta percha point (fig. 167). Osteogenesis was in progress close to the point. An area of inflammation persisted adjacent to the opening of a lateral canal and some resorption of the cementum had occurred. The root apex was not free of bacteria. Colonies of bacteria were seen near the apex (fig. 169) and some organisms had invaded the tubules adjacent to the main canal (fig. 168).

While it is possible that Ledermix paste aided periapical reparative processes in this instance, it did not completely allay inflammation and was unable to eradicate all bacteria, especially those in sites protected from diffusion of the paste.

Fig.163

Fig.164

Fig.165 x12

FIG 167 x 20

FIG 168 x 560
GRAIN

FIG 169 x 560
GRAIN

Fig.166

Experiment 40. Figs. 170 - 173, page 150.

In this experiment, both apices of the lower left 4th premolar were perforated and Ledermix paste was inserted into the canals for a period of one month. Paper points, impregnated with a culture of streptococcus viridans, were then inserted into each canal for a further one month. The object of this experiment was to ascertain whether the effect of the Ledermix paste was sufficiently long lasting to prevent development of an apical reaction and whether the residual paste would sterilise the paper point.

Mesially the paper point extruded slightly into the periapical tissues but no inflammation supervened (fig. 172). This was considered of interest, for sterile and infected paper points were seen to be very irritating to periapical tissues as a general rule in other experiments (pages 16,17.) In this experiment, connective tissue had grown into the canal alongside the paper point in the apical third of the canal (fig. 172). In deeper sections, the root apex appeared to be eroded and replaced by connective tissue growing into the main canal (fig. 171). The mesial aspect of the mesial root was anchylosed and this was commonly seen following perforation of a root apex (fig. 170). Bacteria were located by Gram stain in one area only and this was in contact with the ingrowing connective tissue (a, fig. 171). No inflammatory reaction occurred at this site however.

Distally, a circumscribed acute abscess developed on the distal aspect of the root, but the periapex showed very slight inflammation elsewhere (fig. 170, 173). Both aspects of the root tip were anchylosed. The distal root was negative to Gram stain.

In this experiment, it seems possible that the anti-inflammatory effect of the Ledermix paste had counteracted the irritent action of the infected paper point extruded from the mesial canal. The fact that bacteria were detected in the mesial root in contact with healthy connective tissue without any sign of inflammation may indicate that the defence mechanisms of the periapical tissue had been depressed in this area. The small abscess distally may have been due to the paper point or to undetected bacteria, but the extent of the reaction is small when compared with the results from other experiments using paper points (vide supra).

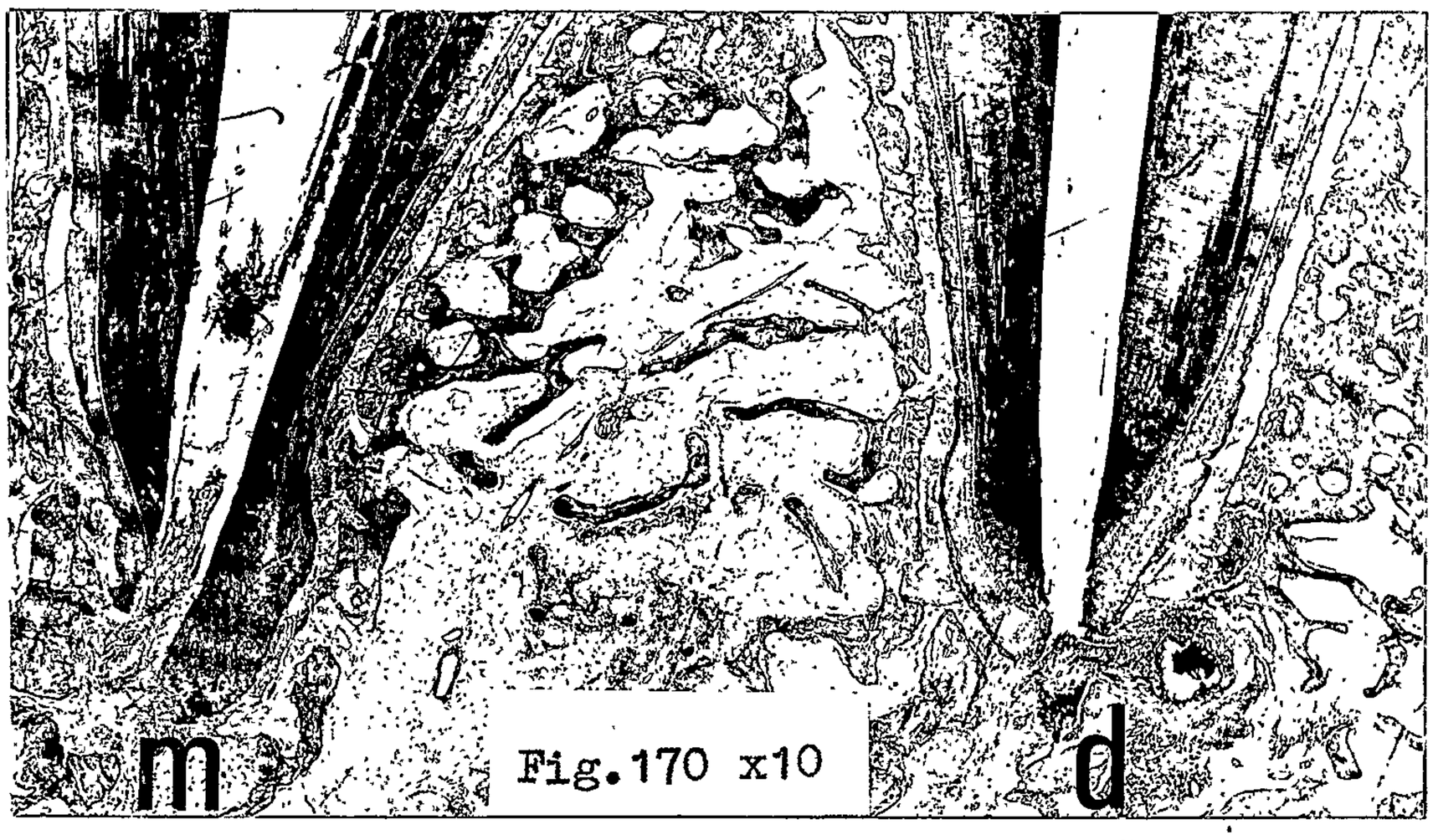


Fig.170 x10

Fig.171 x30

Fig.172 x30

Fig.173 x32

Experiment 41. Figs. 174 - 177, page 152.

Streptococcus viridans, inserted into the canals of the lower right 4th premolar (a, fig. 174) for a period of three months, had produced areas of reaction over both root apices. The mesial canal was sealed off from the distal with amalgam and Ledermix paste was inserted into the distal canal for a period of three months.

Histologic sections showed that inflammation had completely subsided and the distal area had been filled in with loose immature connective tissue (figs. 175, 176). Bone formation appeared suppressed. A few small trabeculae were seen but they were surrounded by dense connective tissue and there were no peripheral osteoblasts (fig. 177). Older trabeculae circumscribing the area had also lost the surface layer of osteoblasts. Gram staining did not reveal bacteria in the distal apex, but mesially, the main canal and a few ramifications were sparesly populated by organisms. The area of bone resorption around the mesial apex was extensive and the inflammatory reaction intense (fig. 175).

In this case, the Ledermix paste appeared to have inhibited osteogenesis following on marked anti-inflammatory activity. In vitro experiments demonstrated that streptococcus viridens is very sensitive to the action of the tetracycline contained in Ledermix paste, and the negative reaction to Gram stain in the distal apex suggested that the drug inhibited proliferation of the organisms in this instance in vivo.

Experiment 42. Figs. 178 - 1818, page 153, 154.

It was the object of this experiment to examine the long term effect of Ledermix paste and Cortril on the periapical tissues. Pulpectomy was carried out in each canal of the lower right third premolar (fig. 178), and the distal canal was sealed off with gutta percha as control. Ledermix paste was inserted into the mesial canal and allowed to remain 3 months. After this period, the radiograph showed an intact lamina dura circumscribing each apex (fig. 178).

Examination of the distal control apex revealed that vital tissue had persisted in the apical ramifications and the periapical tissues are normal (fig. 179).

Pulp remnants in the mesial canal indicated that pulpectomy was

FIG 175 X 6

Fig. 174.

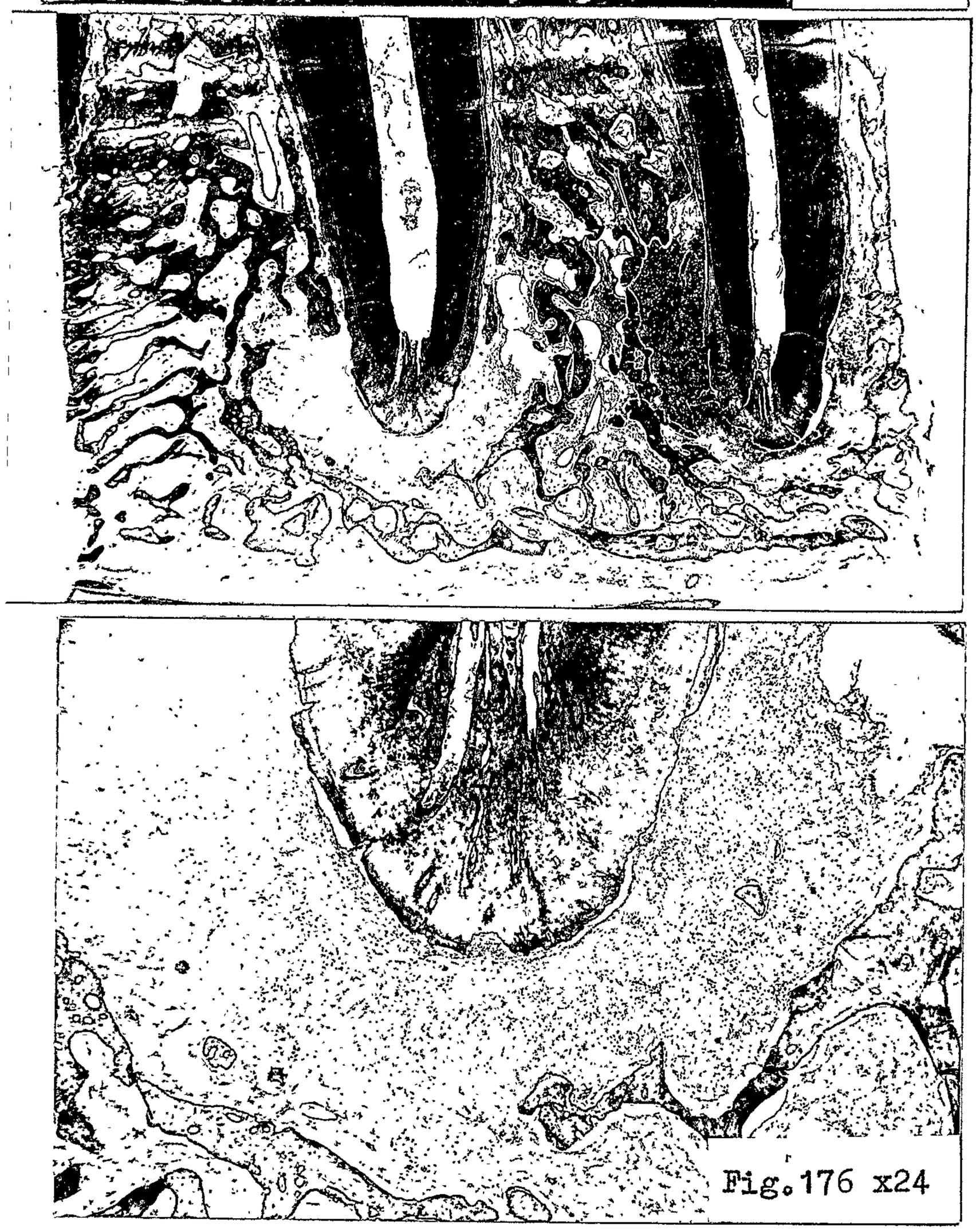


Fig. 176 x24

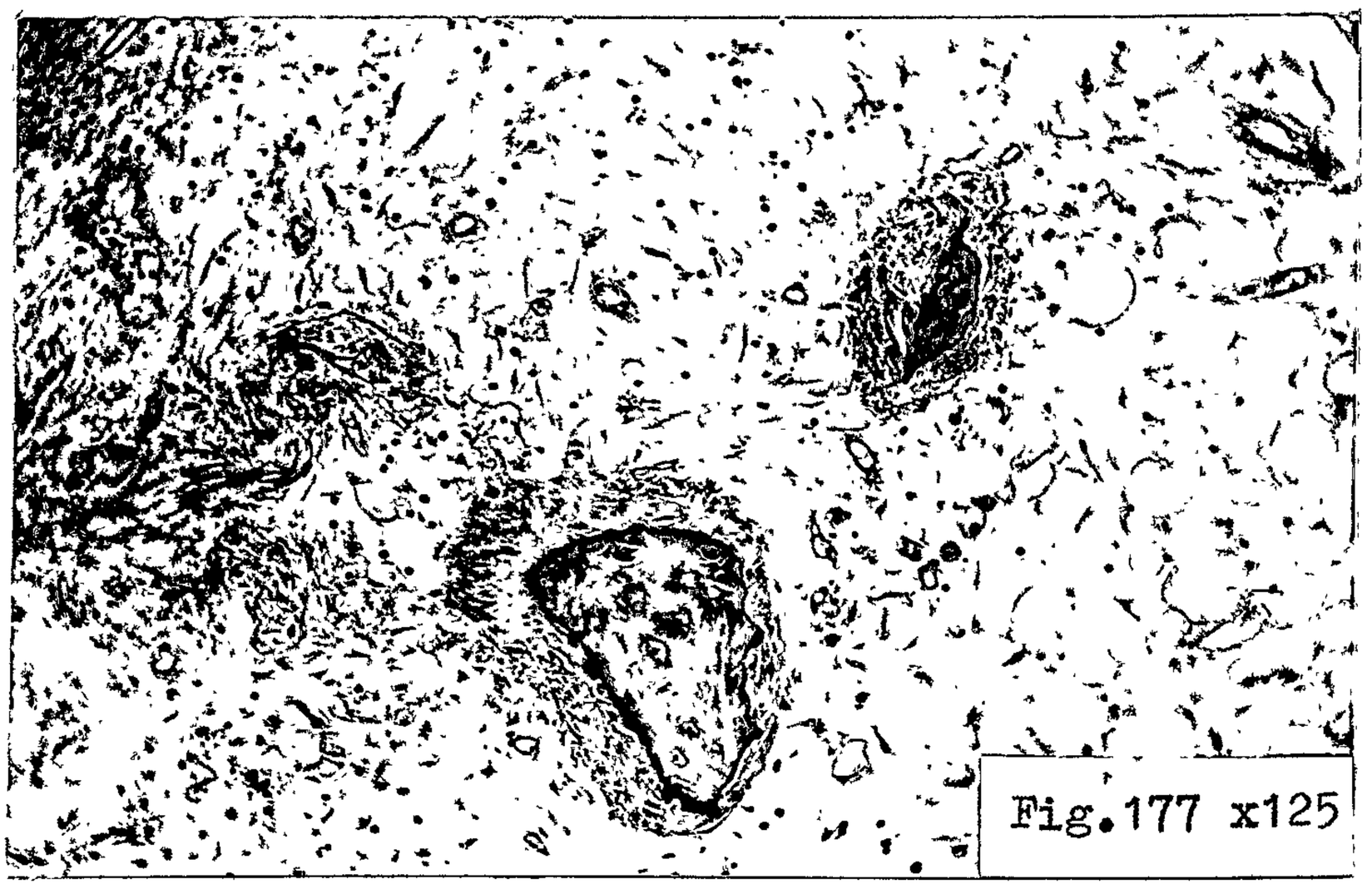


Fig. 177 x125

Fig.178

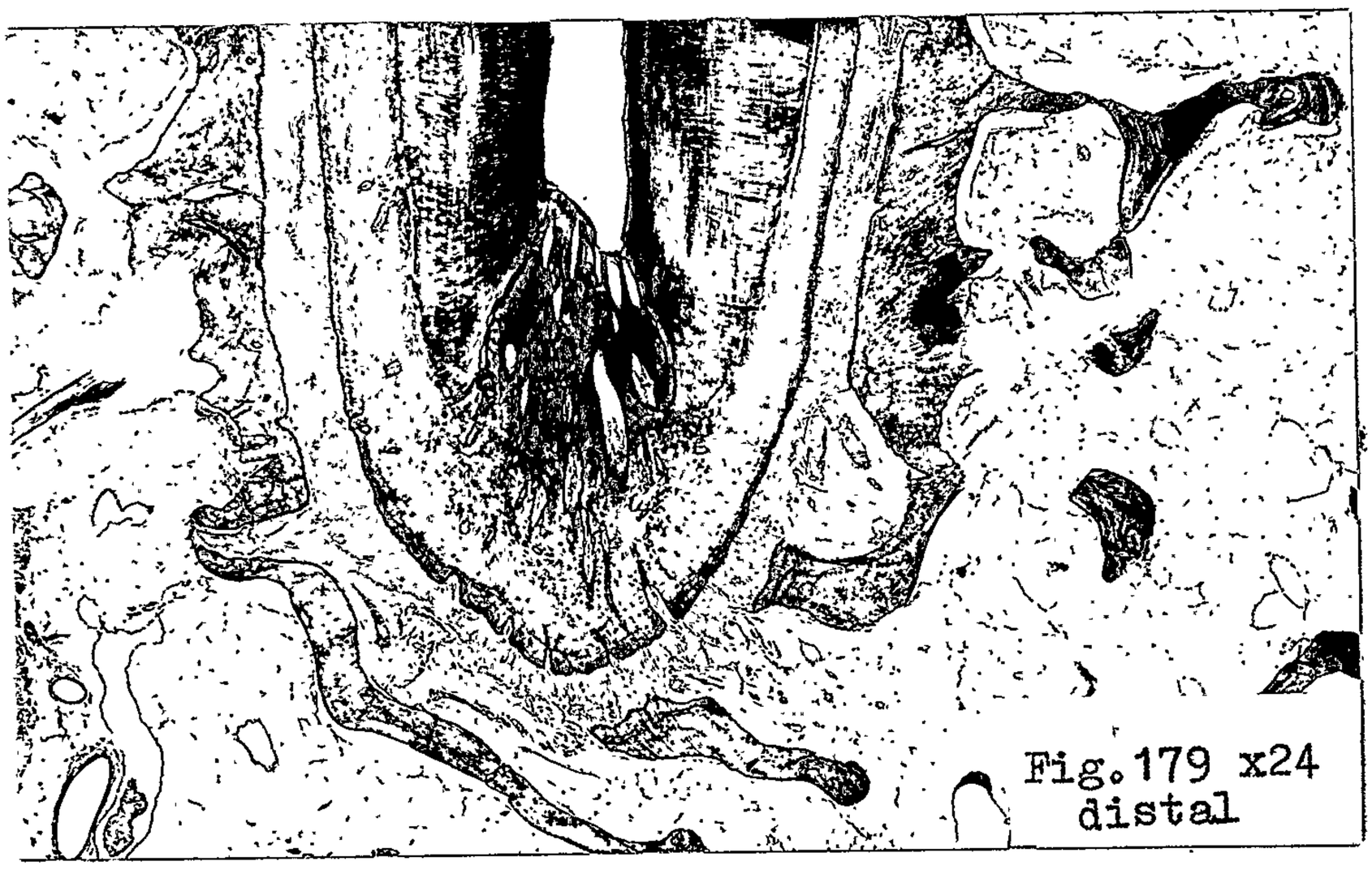


Fig.179 x24
distal

Fig.180 x125

Fig.181 x17
mesial

167 (Similar to F3)

168 (? IK17)

169.

Fig 181A

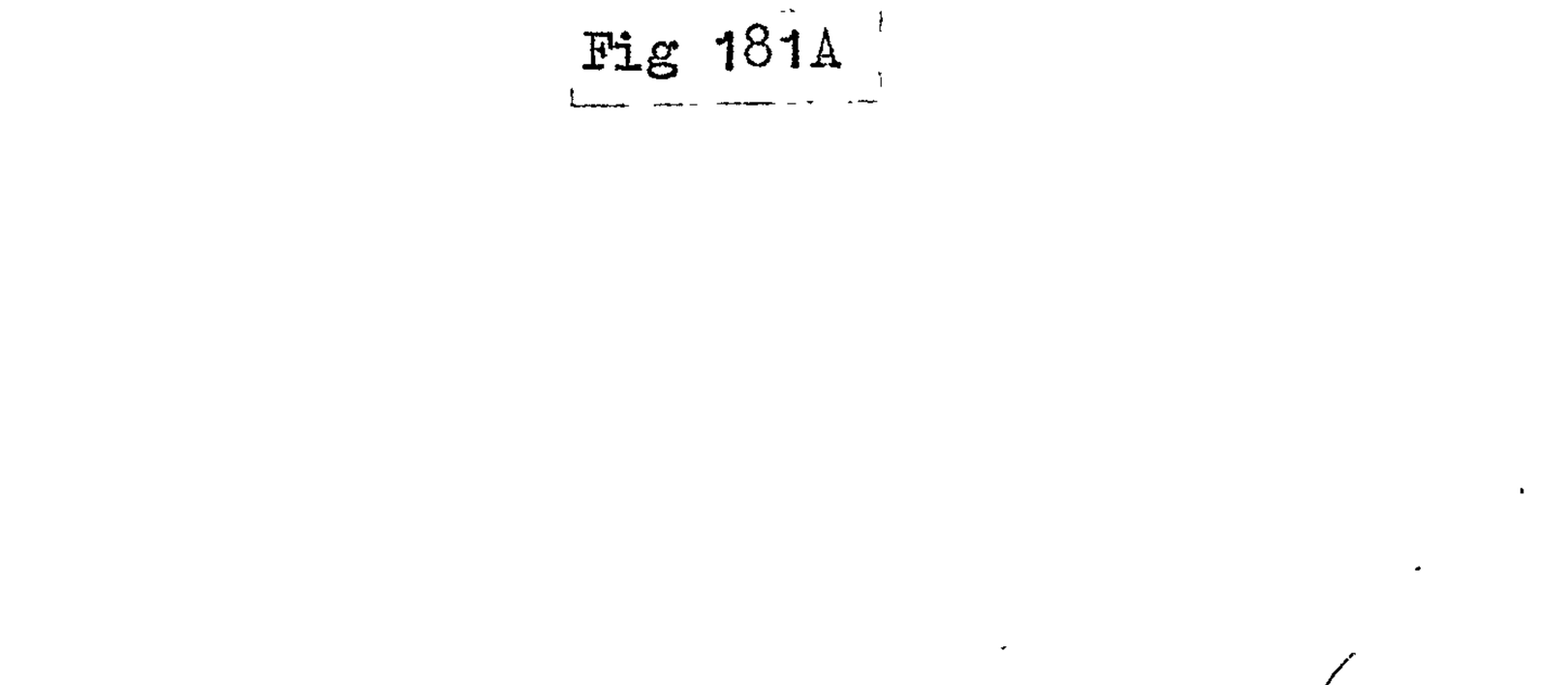


Fig 181B x10

incomplete (fig. 181). Autolysis had not occurred, and in one area a layer of odontoblasts remained intact and in another whorls of pulp tissue retained cell outlines (fig. 180). The periapical bone was intact and the periodontal membrane uninterrupted. In this experiment, Ledermix paste had no effect on healthy periapical bone over a long period.

In another animal, pulpectomy was carried out in both canals of the lower left premolar and Cortril inserted. Radiographs taken after 4 months showed that large areas of rarefaction had developed around the apices, especially distally (fig. 181A). Sections illustrated the development of an extensive inflammatory focus around the distal apex and this contained areas of liquefaction necrosis. The apical reaction was less severe around the mesial apex (fig. 181B). This intense inflammatory response to a paste containing hydrocortisone is difficult to interpret. It is possible, however, that some ingredient in the water soluble vehicle acted as an irritant and counteracted the effect of the hydrocortisone. Experiment 34 instances two teeth in different animals where application of Cortril to exposed pulps resulted in pulp necrosis and apical abscess formation.

Experiment 43. Figs. 182 - 185, page 156.

After three months, streptococcus viridans had produced areas of considerable extent around both root apices of the lower right 3rd premolar (a, fig. 182). The tooth was mobile at the second visit. The mesial canal was sealed off with amalgam and the distal canal reamed and filed through the apex. A culture taken prior to canal cleaning was positive, while a second culture after debridement yielded no growth. This does not necessarily indicate that all bacteria had been eradicated from the root but does illustrate how biomechanical cleansing can substantially reduce the bacterial population in the main canal. Ledermix paste was inserted into the distal canal for a period of two weeks. Following this treatment the paste was irrigated out of the canal and the canal root filled with gutta percha points and root canal sealer. Tooth mobility had been eliminated following treatment with the paste.

Four months later, the area was seen to be much reduced on the

Fig.182

Fig.183

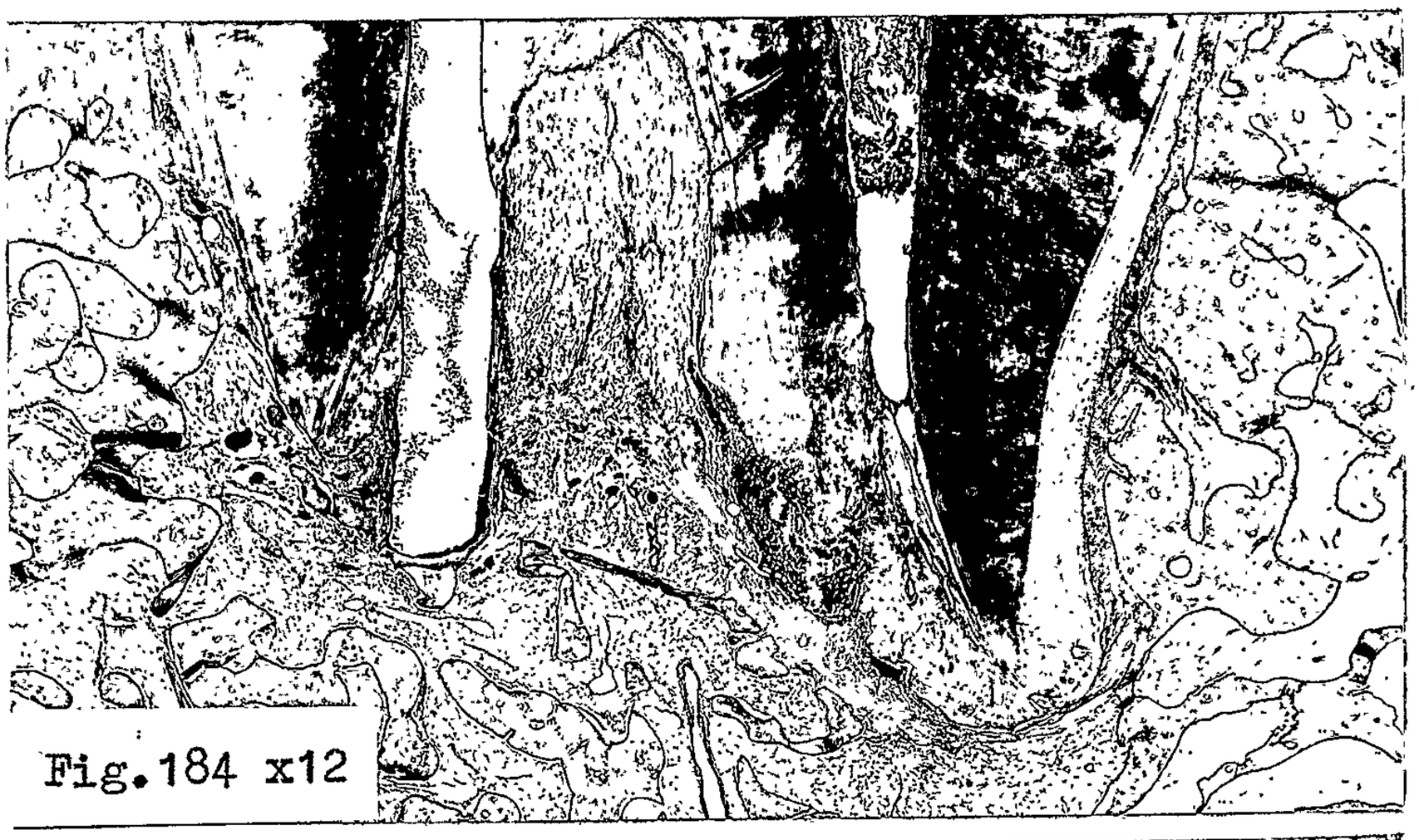


Fig.184 x12

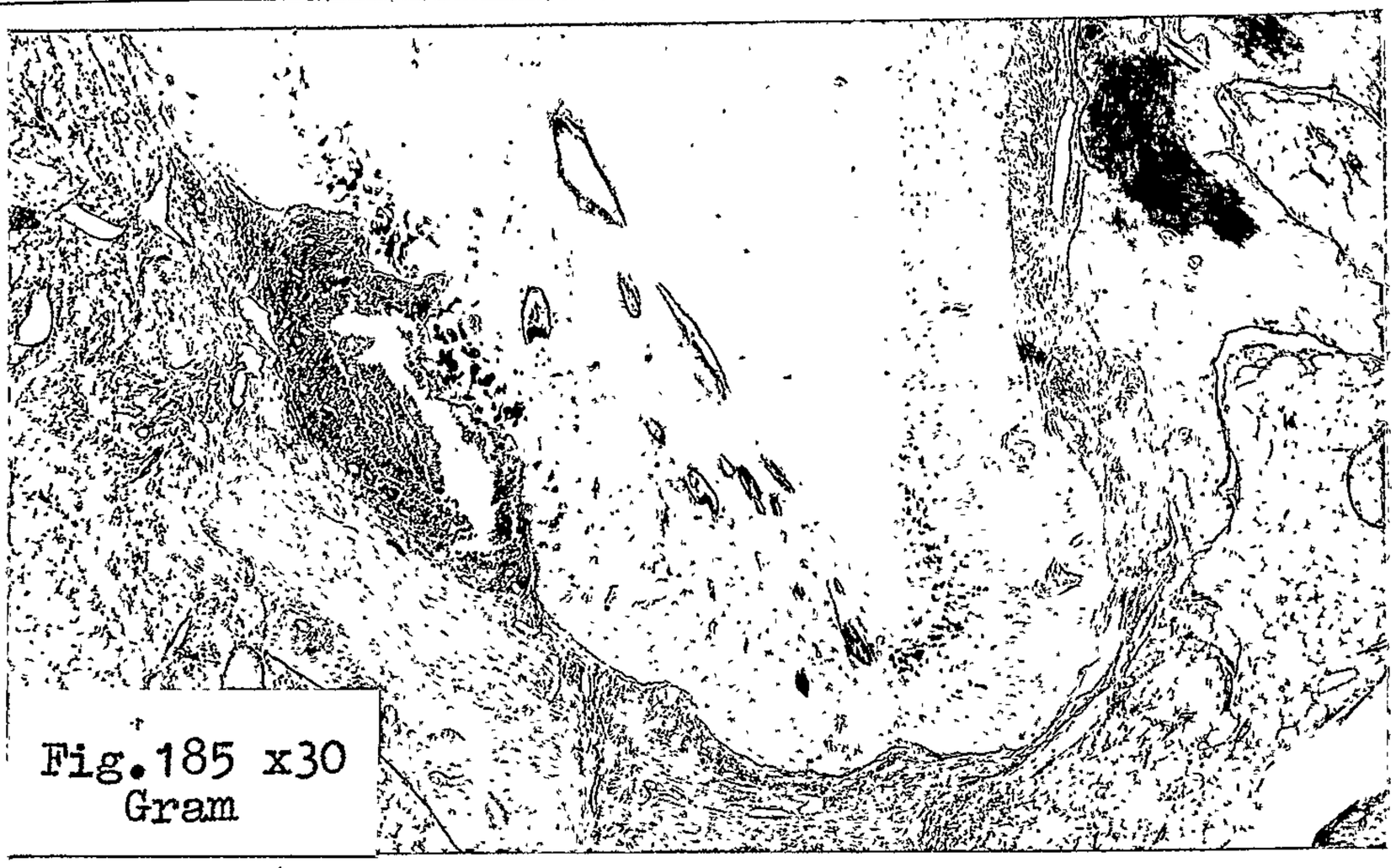


Fig.185 x30
Gram

radiograph (fig. 183), and sections of the tooth showed complete healing around the distal apex (fig. 184). The area of bone resorption was still apparent, but it was filled in with loose connective tissue and newly forming trabeculae. Inflammation still persisted around the mesial root apex, and Gram staining revealed that this apex was heavily colonised with bacteria. Some root resorption had occurred in an area of particularly heavy bacterial infiltration (fig. 185).

In this experiment, the combination of mechanical preparation of the root canal, treatment with Ledermix paste and root filling had resulted in complete subsidence of the inflammatory reaction.

Experiment 44. Figs. 186, 187 - page 158.

Streptococcus viridans had been used to produce areas on both root apices of the lower right 4th premolar. The mesial canal was sealed off with amalgam, and the distal canal reamed and filed to the commencement of the apical ramifications. Ledermix paste was then inserted into the distal canal for two weeks. After this period root filling with root canal sealer and gutta percha was carried out.

A radiograph obtained 4 months later suggested that healing had occurred around the distal apex (fig. 186) and this was confirmed by examination of serial sections (fig. 187). An area of reaction of substantial size persisted around the untreated mesial root apex.

While it has been shown elsewhere that mechanical preparation of experimentally infected canals followed by root filling promotes apical healing in a proportion of cases it is possible that the additional treatment with Ledermix paste contributed in this case to the suppression of bacterial activity and aided resolution in the periapical tissues.

Experiment 45. Figs. 188, 189, page 158.

After 2 months, streptococcus viridans had produced areas of reaction on both root apices of the lower left third premolar (fig. 188). The mesial canal was sealed off from the distal with amalgam, and the distal apex perforated. Ledermix paste was then spun through the apex with a Lentulo spiral instrument. The animal was sacrificed two months later.

Fig.186

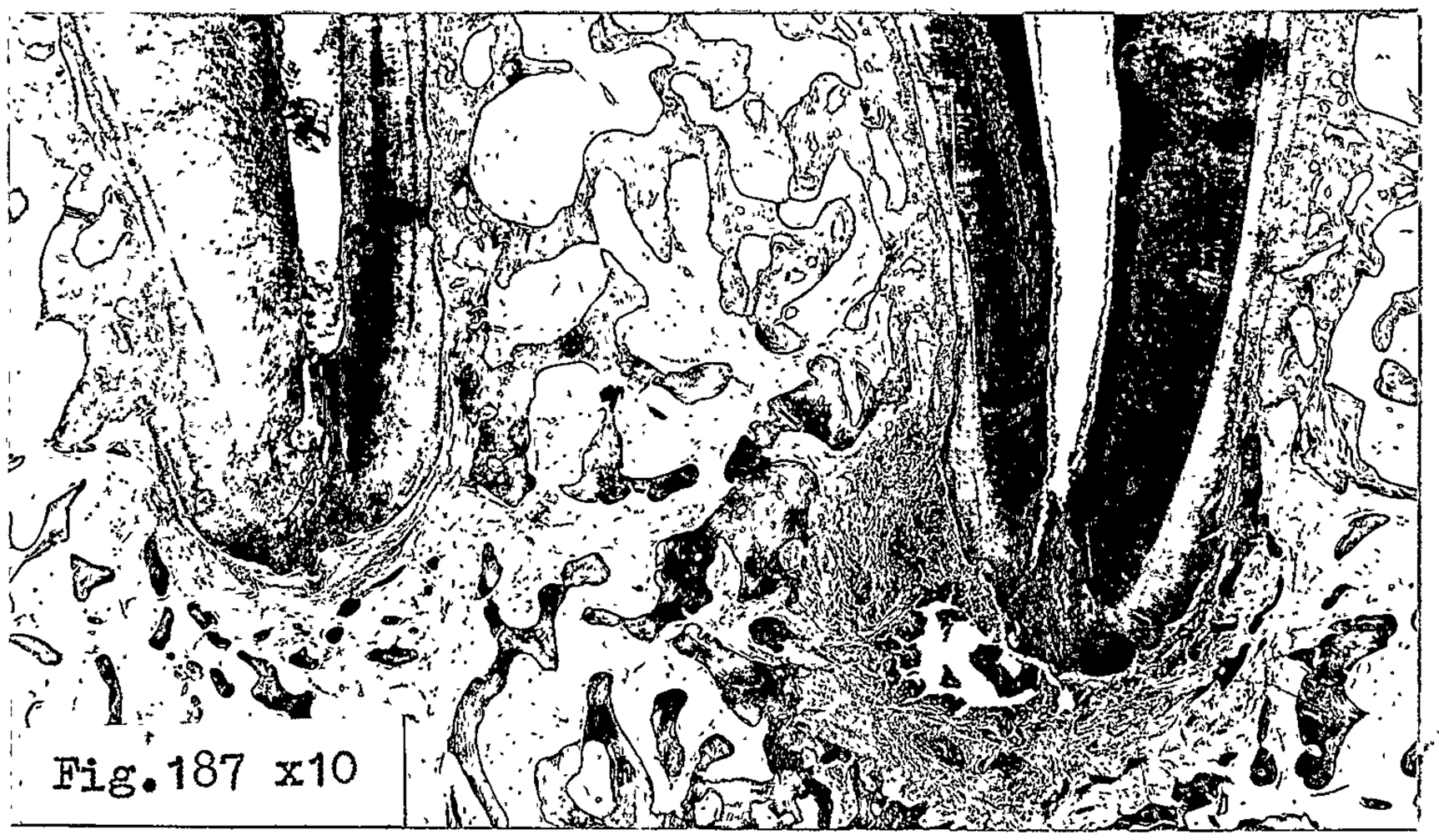


Fig.187 x10

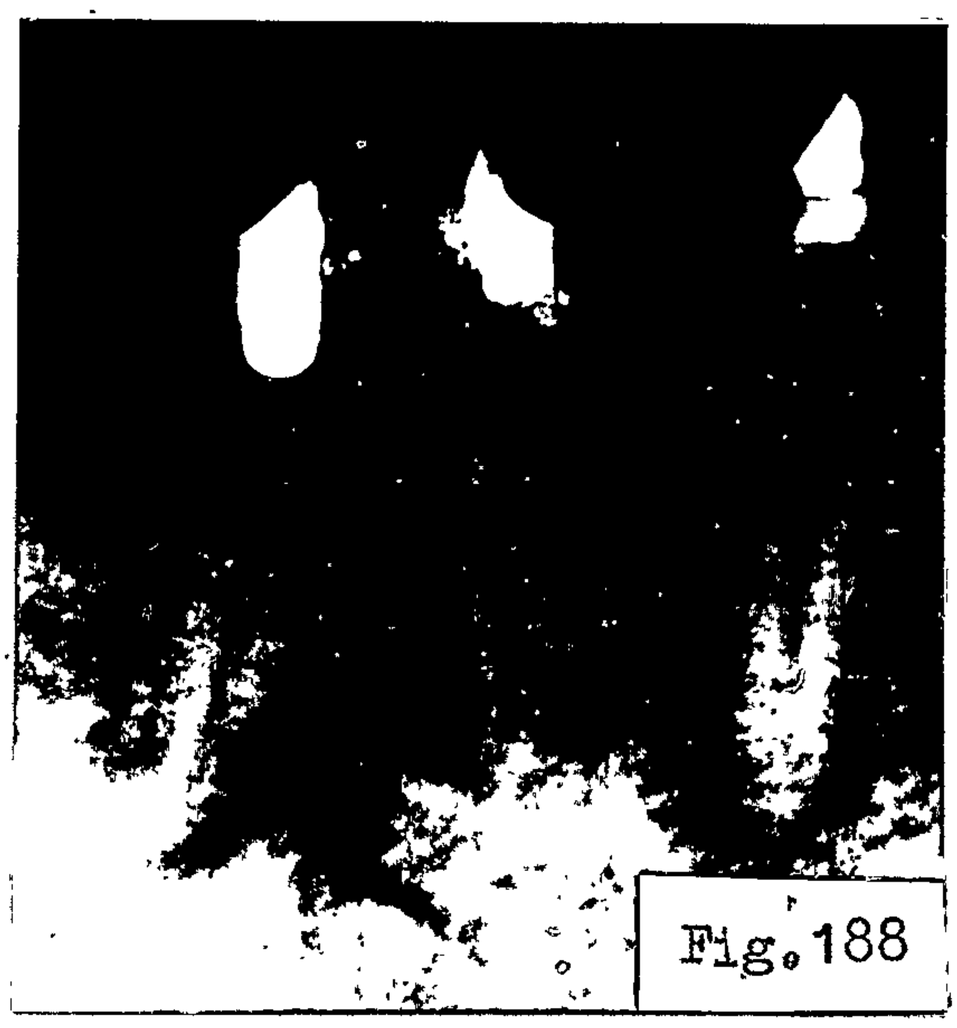


Fig.188

Fig.189 x10

Histologic sections showed soft tissue extending for a short distance into the apical perforation and this tissue was free from inflammatory cells (fig. 189). Portion of the root tip had fractured but neither resorption nor anchylosis had occurred. Foci of inflammatory cells persisted around the mesial apex.

Resolution of apical inflammation around the distal apex may have been due in part to the effect of the corticosteroid/antibiotic compound and this compound did not appear to hinder the reconstruction of apical bone. The ingrowth of healthy connective tissue into the hollow canal is interesting. The work of Dixon et al[64] demonstrated the development of an inflammatory reaction around the orifice of hollow tubes embedded in tissue, and a similar response around the perforated apex of an empty root canal would not have been unexpected. The investigations by Ostby[185], however, have shown that in favourable circumstances, blood clot in the apical portion of a canal from which the pulp has been removed may become organised with extension of periodontal tissue for a limited distance into the hollow canal without coincident inflammation. Evidently treatment of the canal in this experiment with the corticosteroid/antibiotic compound provided a suitable environment for the occurrence of this latter event.

Experiment 46. Figs. 190 - 192, page 160.

Streptococcus viridans caused the development of periapical areas on both roots of the lower left second premolar (E, fig. 190), the area around the mesial apex being considerably larger than that which developed distally. The distal canal was sealed with amalgam while the mesial canal was reamed and filed to the cemento-dentinal junction. Ledermix paste was inserted, and after a period of two weeks, the animal was sacrificed.

A section of the tooth in situ confirmed the small size of the distal area and it will be noted that an inflammatory reaction was still present around the mesial root apex (fig. 191).

There appeared to be a marked diminution in the extent of this mesial reaction when the histologic picture was compared with the size of the area depicted on the radiograph taken two weeks earlier.

M. D.

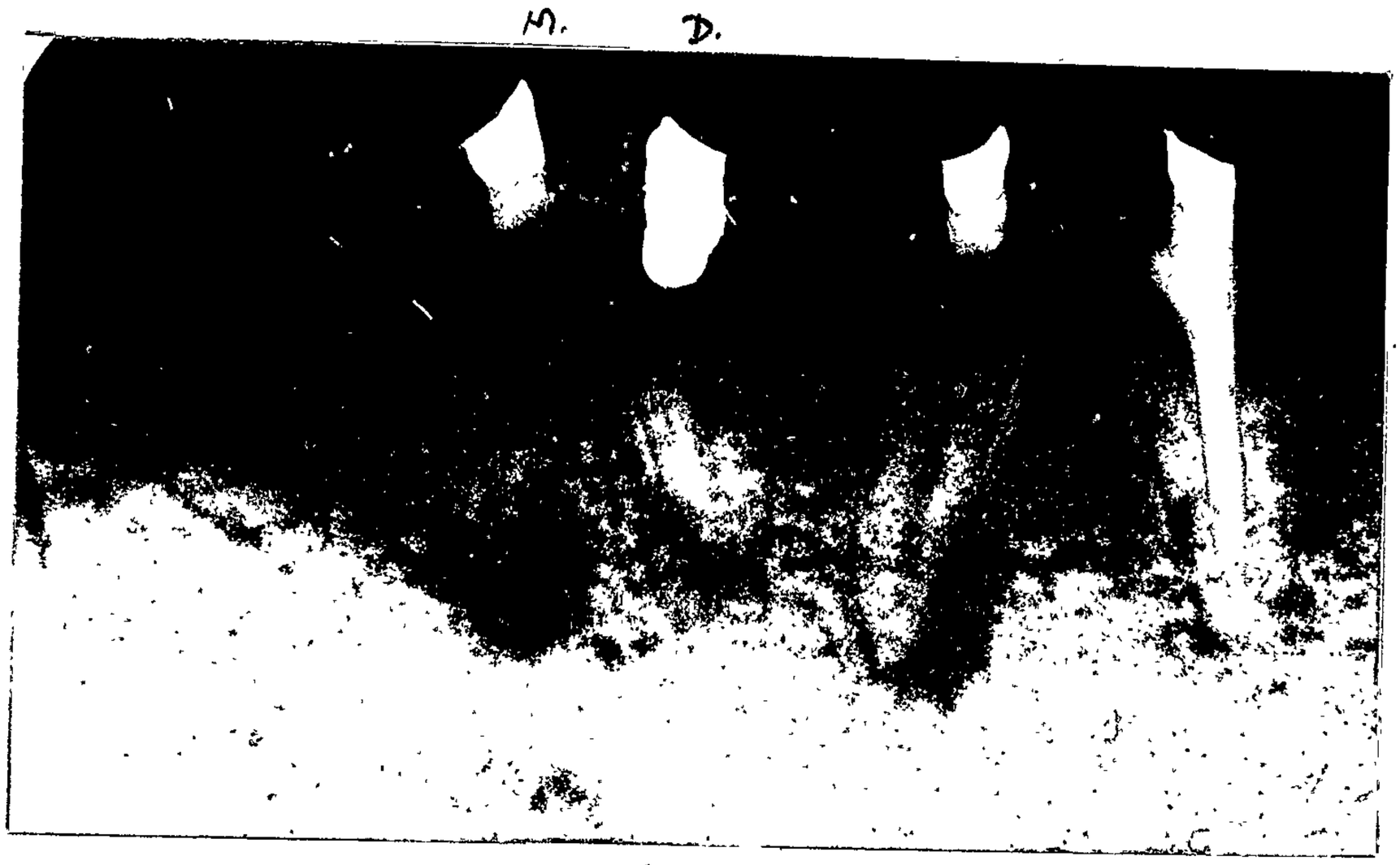


Fig. 190

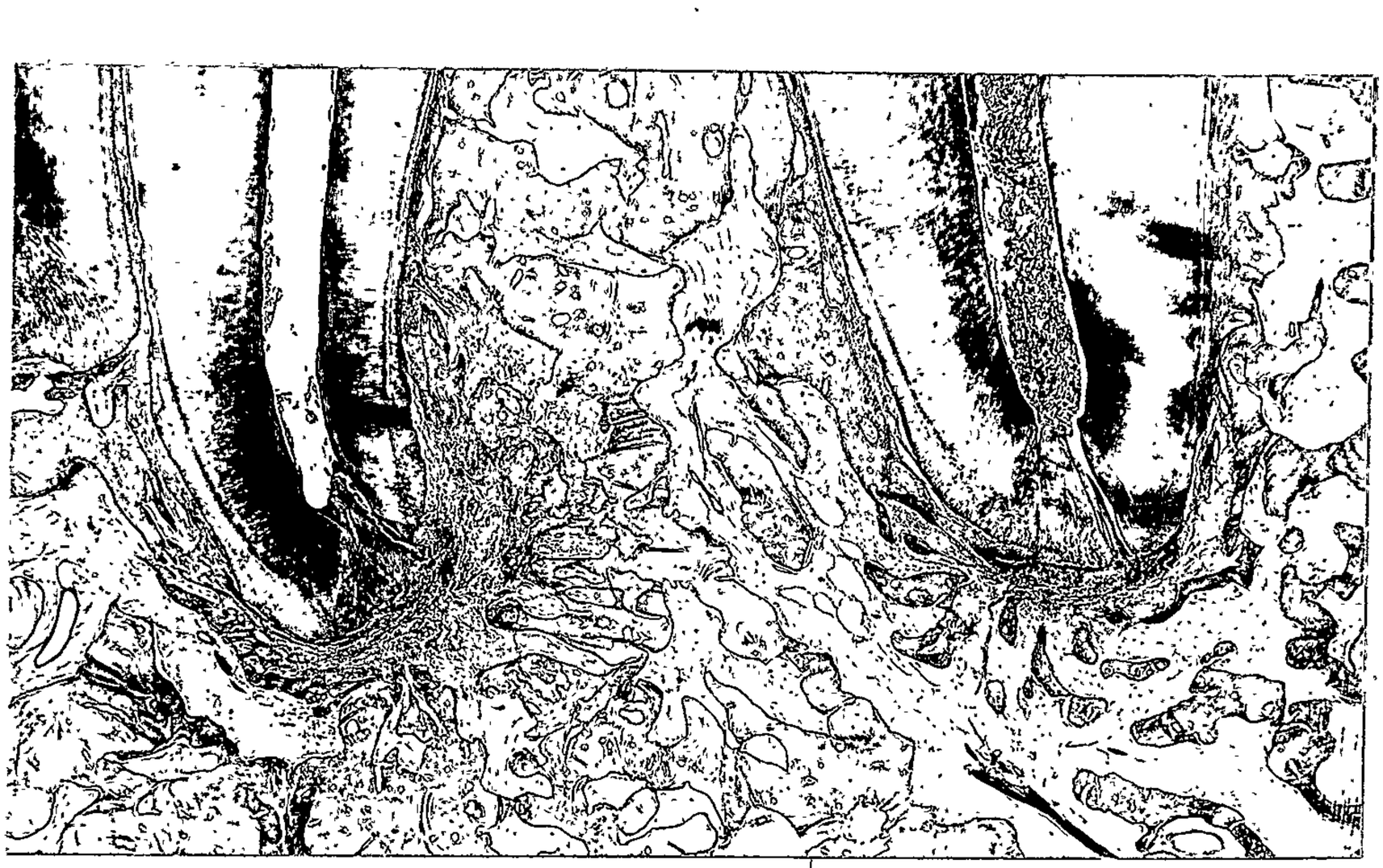


Fig. 191 x11

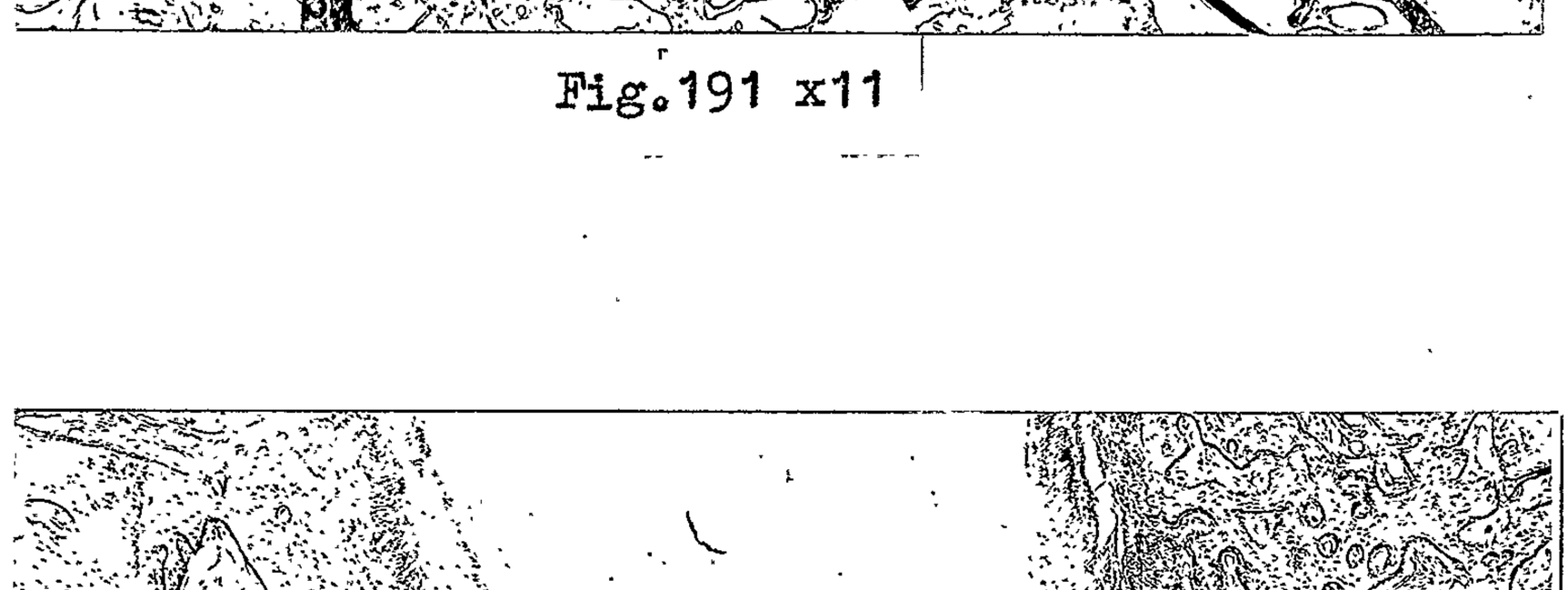


Fig. 192 x28
Gram

Gram stain indicated that the mesial root tip continued to support a bacterial population (fig. 192).

In this case, Ledermix paste failed to eradicate bacteria from the cemental lacunae and the inflammatory reaction in the periapical tissues persisted. It is possible however that some attenuation of the reaction resulted from the treatment.

Experiment 47. Figs. 193 - 195, page 162.

Inoculation of the mesial and distal canals of the lower left 4th premolar (e, fig. 193) with streptococcus viridans had produced areas over both root apices after 8 months. The distal canal was sealed off with amalgam and the mesial apex was perforated. Ledermix paste was inserted into the mesial canal for two weeks.

Sections showed areas of reaction over both apices, more vigorous mesially (fig. 194) than distally (fig. 195). The perforated mesial canal was surrounded by a severe inflammatory reaction and leucocytes, predominately of the polymorphonuclear variety, extended into the canal. In this case there was no evidence of the anti-inflammatory effect of the paste and Gram staining indicated that bacteria still resided in the lacunae of the mesial apex.

Experiment 48. Figs. 196 - 199, page 163.

The irritant effect of a paper point which had been extruded through a perforated apex into the periapical tissues has been illustrated previously (page 17). In the experiment described below, the reaction of the tissues to a sterile paper point was compared with the disturbance caused by a point impregnated with Ledermix paste, in order to assess the anti-inflammatory potential of the corticosteroid compound in this situation.

The two root apices of the lower left 4th premolar (e, fig. 196) were perforated and then paper points inserted into both canals, the distal canal having been filled initially with Ledermix paste.

After 6 months, sections revealed that the mesial point had caused extreme apical irritation with root resorption (fig. 197) and the inflammatory reaction enveloped the whole of the extruded point. Distally, there was a circumscribed area of inflammation confined to the

Fig.193

Fig.194 x25
mesial

Fig.195 x25
distal

Fig. 196

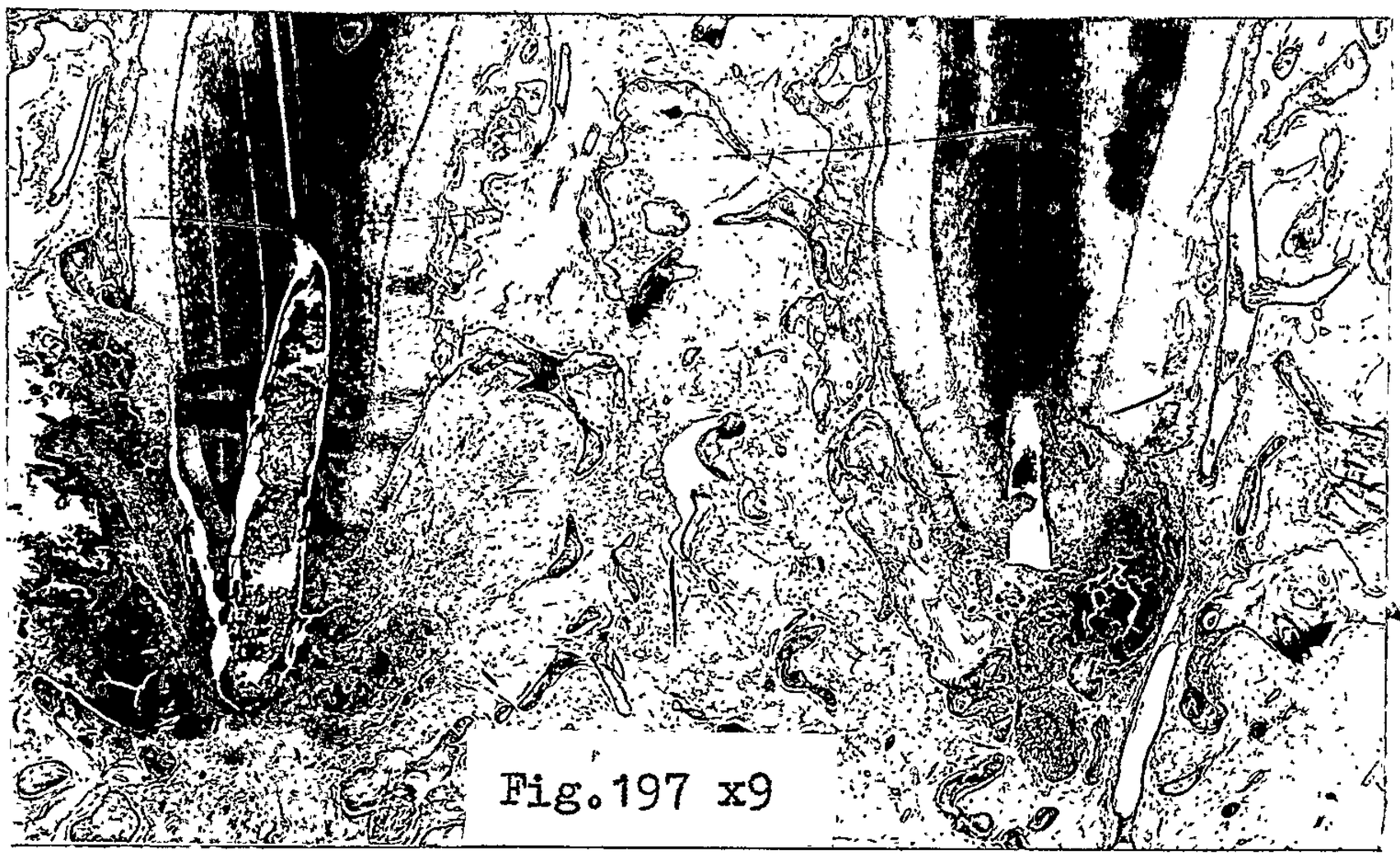


Fig. 197 x9

Fig. 198 x25

Fig. 199 x25

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distal aspect of the paper point, but elsewhere the point was in contact with bone and connective tissue unnattended by any reaction (figs. 197,198). In one location (s, fig. 199) there was an area of hyperaemic tissue and resorption of the canal apex was proceeding nearby.

In this instance, Ledermix paste appeared to have partially suppressed the inflammatory reaction which usually supervenes following impingement of a paper point on the periapical tissues. Further experimentation is required to evaluate the anti-inflammatory activity of this compound.

Tooth treated	Treatment	Period of observation	Results
24. LR 2nd Pm	Ledermix cement applied to distal pulp exposure.	4 months	Loss of predentine and odontoblasts coronally and cementum formation in pulp deep to exposure. Pulp otherwise normal and periapex undisturbed.
25. LR 3rd Pm	Ledermix cement applied to exposed mesial and distal pulp.	2 months	Pulp fibrosed with loss of specialised elements.
26. LL 3rd Pm	Ledermix cement applied to distal pulp exposure.	6 months	Pulp degenerated and blood vessels dilated. Ingrowth of very cellular cementum into canal lumen from cemento-dentinal junction.
27. LR 2nd Pm	Ledermix cement applied to distal pulp exposure.	3 months	Loss of predentine and odontoblasts around exposure. Vacuoles coronally but remainder of pulp and periapex intact.
28. LR 2nd Pm	Ledermix cement applied to distal pulp exposure.	8 months	Partial loss of odontoblast layer. Dentine chips free in pulp without any surrounding reaction. Secondary dentine elaborated coronal to exposure. Pulp normal.
29. LR 2nd Pm	Ledermix cement applied to distal pulp exposure.	3 months	Pulp normal but some dilated blood vessels deep to exposure.

30.	LL 4th Pm	Ledermix cement applied to distal pulp horn.	3 months	Pulp normal except for some dilated vessels. Dentine chips encased in hard tissue and attached to pulp chamber wall.
31.	LL 2nd Pm	Ledermix cement flowed over pulp exposure.	3 months	No reaction in pulp; dentine chips lie free; some blood vessels dilated; partial loss of odontoblasts.
32.	LR 3rd Pm	Ledermix cement deeply inserted into pulp exposure.	4 months	Loss of odontoblasts and predentine layer near exposure. Small area of dentine resorption near cement; pulp otherwise normal.
33.	LR Pm	Ledermix cement flowed over exposure.	8 months	Pulp normal apart from some dilated vessels.
34a	LR 2nd Pm	Ledermix paste applied to distal exposure; cortril over mesial exposure.	2 months	Loss of cellular elements in pulp beneath Ledermix paste; periapex normal. Complete pulp destruction beneath cortril with acute apical abscess formation.
34b	LL 2nd Pm	Cortril applied to pulp mesially and distally.		Pulp degeneration and acute apical abscess formation mesially and distally.
35.	L Pm	Ledermix paste applied to mesial and distal pulp.	1 month	Degeneration of pulp in each canal. Periapical region normal.
36.	L Pm	Ledermix paste on cotton wool applied to pulp.	6 months	Pulp vital; some dentine chips encased in hard tissue and attached to canal wall.

37.	LR 3rd and 4th Pm	Streptococcus viridans used to develop areas on all four apices. Ledermix paste inserted into canals of LR 4th premolar for 2 weeks. All 4 canals then root filled.	4 months	Apart from some small foci of inflammatory cells around each apex, healing was in progress. Ledermix paste did not completely eradicate bacteria from cemental lacunae and ramifications.
38.	LR 4th Pm	Streptococcus viridans used to produce areas on both apices. Ledermix paste mesially; canal sealed distally.	3 months	Some residual inflammation on each apex although bone cavities mainly filled in with connective tissue. Some new trabeculae forming, but little difference between progress of healing mesially or distally. Ledermix paste did not induce complete subsidence of inflammation.
39.	LL 4th Pm	Streptococcus viridans produced areas on each apex. Ledermix paste and GP points in each canal following perforation of the apex.	2 months	Some residual inflammation although area of reaction had markedly decreased and bone formation was in progress. Ledermix paste did not eradicate all bacteria from tubules and apex.

40.	LL 4th Pm	Apices perforated and Ledermix paste inserted for 1 month. Paper points with streptococcus viridans inserted.	1 month	Mesially, extruded paper point caused no reaction and connective tissue ingrowth seen alongside paper point. Bacteria in canal caused no cellular disturbance. Distally, a small circumscribed abscess developed.
41.	LR 4th Pm	Apical areas produced by streptococcus viridans. Mesial canal sealed off and Ledermix paste inserted into distal.	3 months	Complete subsidence of inflammation distally; area filled in with loose connective tissue. Bone formation suppressed. Distal apex negative to Gram stain.
42a	LR 3rd Pm	Pulpectomy in each root. Distal control; Ledermix paste inserted mesially.	3 months	Periapical tissues normal around both apices. Ledermix paste had no discernible effect on healthy periapical tissue.
42b	LL 4th Pm	Cortril inserted into each canal following pulpectomy.	4 months	Extensive inflammatory reaction, especially around distal apex.
43.	LR 3rd Pm	Areas produced by streptococcus viridans. Mesial control; distal canal perforated and enlarged (culture negative); Ledermix paste	4 months	Complete healing around distal apex due to combination of mechanical preparation, Ledermix paste and root filling.

		inserted for 2 weeks. Then root filled.		
45.	LL 3rd Pm	Areas produced by streptococcus viridans. Mesial control. Distal apex perforated and Ledermix paste inserted.	2 months	Connective tissue ingrowth for short distance into distal canal. Foci of inflammatory cells persist around mesial apex.
46.	LL 2nd Pm	Areas produced by streptococcus viridans. Distal control. Mesial canal enlarged to C.D. junction and Ledermix paste inserted.	2 weeks	An apparent reduction in size of the mesial area in this brief period. Bacteria still resident in mesial apex. Anti-inflammatory and antibacterial activity of Ledermix paste only partially effective in this case.
47.	LL 4th Pm	Areas produced by streptococcus viridans. Distal control. Mesial apex perforated and Ledermix paste inserted.	2 weeks	Reaction more vigorous around mesial apex than distal. Bacteria still present in both apices. Ledermix paste ineffective in this case.

48.	LL 4th Pm	Both apices perforated. Sterile paper point inserted mesially; Ledermix impregnated point distally.	6 months	Sterile point mesially has caused extensive reaction. Reaction to Ledermix impregnated point confined to small area; elsewhere connective tissue and bone in contact with paper point.

Discussion

Ten teeth in different dogs were used to investigate the reaction of exposed pulp tissue to Ledermix cement and it is of interest to compare the results with those from human studies. After observation periods ranging from 2 to 8 months, eight of the ten pulps were judged to be normal throughout most of their extent. Human studies do not refer to any instance of pulp degeneration following application of the material to healthy tissue and describe only slight changes in the soft tissue. Loss of the odontoblast layer and the underlying predentine zone from the walls of the pulp chamber in the vicinity of the exposure site occurred in five of the dogs' teeth, and dilated blood vessels were noted under the cement in several of the treated pulps. These changes have been commonly observed in human teeth. In one case (expt. 27, e, fig. 135) pronounced basophilia accompanied the commencement of the odontoblast layer and this again indicates that the response of dog pulp resembles the changes seen in human tissue. Reports are not uniform with regard to the incidence of hard tissue formation in treated human pulps although after prolonged periods it does seem that a moderate calcific response may occur in some pulps beneath the exposure site. In the dog, newly formed hard tissue occurred infrequently and dentine bridging was not observed. A small amount of amorphous calcified tissue formed on the canal wall in two teeth, and dentine chips were encased in one instance. An unusual occurrence occurred in one pulp where cellular cementum-like tissue formed around dentine chips and gained attachment to the canal walls. This reaction has not been observed in human specimens. Although the cement had been forced into the pulp tissue in some of the dogs' teeth there was no evidence of soft tissue compression, inflammation or walling off with fibrous tissue. One of the eight pulps (expt. 27) classed as successfully treated, did show some vacuole formation and this was probably not artifact.

The two failures must be considered. In one case (expt. 25), deep pulpotomy cavities had been cut mesially and distally and both pulp canals then covered with the cement. This operative procedure may have completely disrupted the anastomosis of blood vessels across

the coronal area resulting in the degeneration of the pulp to a fibrous remnant. The possibility that pulp degeneration was caused by the cement cannot be entirely excluded however. In the second case (expt.26) pulp disorganisation was possibly the result of artifact due to inadequate fixation, but the ingrowth of a column of very cellular cementum into the apical canal is of particular interest and warranted a full report. Further investigation is required to ascertain whether this unusual phenomenon can be attributed to the effect of the corticosteroid cement. In both the above cases, the periapical tissues remained undisturbed.

From this comparison, it may be seen that the range of reaction of dog pulp to Ledermix cement is basically similar to the variation in response observed in human pulp. Dog pulp appears to be more prone to degeneration following injury than human pulp, and the fact that several teeth treated with this material retained their vitality may attest to the tissue compatibility of Ledermix cement in the human situation. The occurrence of cementum and the occasional formation of vacuoles in treated pulps appears, in the present state of our knowledge, to be peculiar to the dog.

Prolonged contact of Ledermix paste with pulp tissue is not recommended by the manufacturers, although some investigators did not observe deleterious results in human pulp tissue following periods of application exceeding one month. In one dog experiment (expt. 36) the pulp remained vital and dentine chips became encased in hard tissue, while in two other teeth pulp degeneration occurred. This may have been partially due to the trauma resulting from cutting deep pulpotomy cavities mesially and distally as mentioned above and it is recommended that future experimentation in dogs' teeth should involve superficial exposure of the pulp at one location only. See p157 The periapical region over all roots treated with Ledermix paste remained normal and this is in contrast with the extensive abscess formation which resulted from the treatment of pulps in three root canals with Cortril. This hydrocortisone compound, mixed in a supposedly bland water miscible base, caused pulp death and severe apical irritation whenever it was used in dogs' teeth, but the reason why its effect was completely

contrary to the anti-inflammatory action expected is unknown.

When Ledermix paste was used in canals where apical areas of reaction had been produced by streptococcus viridans, the results were variable. In one experiment (41), the effect of the corticosteroid/antibiotic paste on inflamed periapical tissue was dramatic. After 3 months there was complete subsidence of inflammation, the bone cavity had been filled in with immature connective tissue, and bone formation had been suppressed. This retardation of reparative processes did not occur in other experiments. In a similar experimental situation (45), where the apex had been perforated prior to inserting the paste, inflammation subsided and connective tissue entered the apical opening of the canal. Two other instances of successful healing were observed (expts. 43, 44) when treatment with Ledermix paste for 2 weeks had been followed by root filling with zinc oxide based paste. The apparent eradication of bacteria and subsequent apical healing in these instances may have been due to the combination of mechanical preparation, treatment with Ledermix paste and root filling, and it is impossible to state which procedure was of prime importance in attaining healing.

On four occasions, however, treatment of canals with the paste did not suppress the inflammatory reaction and bacteria continued to reside in the root apices (expts. 37,38,39,46). In a further case (expt. 47) perforation of the root apex and treatment of the canal with Ledermix paste for 2 weeks appeared to result in an exacerbation of the inflammatory reaction when compared with the control. The relative intensity of the areas of reaction were admittedly unknown prior to treatment.

These results following use of Ledermix paste in infected dog root canals are equivocal and do not allow definitive comment on the usefulness of this material. In vitro experiments demonstrated that streptococcus viridans is very sensitive to the tetracycline component in the paste, but in five of the nine dog experiments where Ledermix paste was used in root canals infected with this organism, the streptococcus continued to flourish in protected sites in the root apices, even after a prolonged period of action of the drug. In view

of this frequent ineffectiveness in vivo against sensitive organisms, and the fact that various organisms infecting human root canals are in any event insensitive to the tetracycline it is apparent that Ledermix paste is not a panacea for treatment of infected root canals. Its clinical usefulness in alleviating the pain of pericementitis is well attested, but this does not necessarily denote subsidence of periapical inflammation.

When inserted into sterile root canals, Ledermix paste appeared to have no discernible effect on the periapical tissues--this is in marked contrast to the irritation caused by Cortril (expt. 42).

Impregnation of paper points with Ledermix paste appeared to reduce the intensity of the inflammatory reaction when the point was in contact with the periapical tissues (expts. 40, 48).

SECTION 6

Formaldehyde preparations

The use of formaldehyde preperations for the devitalisation and preservation of pulp tissue is an established method of treatment among European colleagues. The classical technique involves the initial devitalisation of the pulp either by arsenic or a paraformaldehyde preparation, followed by removal of the coronal parts, then the covering of the radicular portion with an antiseptic drug, usually a paraformaldehyde paste. This permeates the remaining devitalised tissues and preserves them, it is hoped, without undue irritation of the viable tissues in the apical canal. The object is to preserve the radicular portion of the dental pulp in an aseptic state so as to obviate the necessity for root canal therapy. In favourable circumstances, the apical periodontal tissues seal the foramen by apposition of cementum[40]. Over the years, there has been little published on the method in English journals although from time to time, some authors have queried whether it may not be of use in the treatment of fine canals in multirooted teeth[150,40,145].

The controversy which exists concerning the use of these formaldehyde preperations has been revived in recent years, firstly by Davies[61] who states that pulp mummification is sufficiently successful to warrant its use in deciduous molar teeth, secondly by renewed interest in formocresol treatment of exposed deciduous pulps, and thirdly by the introduction of N2, a paraformaldehyde containing paste which has gained a deal of popularity among clinicians.

Pulp mummification

Witzel is credited with introduction of pulp mummification in 1872 and he reported only 3% failures in 8000 treatments. In 1885 Lepkowsky was the first to recommend formalin for mummification and in 1898 Gysi introduced his Trio paste combining formaldehyde and creosote derivatives, and this paste retained potent antiseptic properties over an indefinite period. Trio paste became very popular as a mummifying agent and is still very widely used[49]. In 1913, however, Grove[101] in an American Journal warned against the use of formaldehyde preparations in view of the irritant nature of

the drug. This was answered by several articles from European dental teachers.

Lutz[151] in 1924 described the effects of Trio paste in 62 teeth which had been in place for a period exceeding 6½ years. In each case the periodontal tissues had remained healthy and when the filling was removed, the odour of medicament remained. He assumed from this that the formaldehyde component was still active. The pulp stumps were tough and leathery and were bacteriologically negative in all 62 cases. Histologically, the apical foramen was filled with connective tissue and narrowed by formation of cementum. In two cases there was complete closure of the apical foramen. This mode of healing is similar to that following vital extirpation.

Castagnola[40] and Kronfeld[139] confirm this picture of healing following the use of Trio paste. There is ingrowth of tissue from the periodontal membrane which subsequently produces cementum.

Hess[118] in 1929 found that the devitalised and mummified pulp remains as a structurally intact tissue without shrinkage. The necrobiotic zone, i.e., the junction of living and dead tissue is composed of granulation tissue with infiltration of lymphocytes and leucocytes. Cementum deposition occurred at the apical foramen, following an initial resorption of the dentine wall. Hess concluded that pulp devitalisation, followed by amputation of the major part of the pulp and mummification of the remainder with Trio paste is sound treatment proven by clinical, bacteriological and histological investigation, provided the pulp operated on is healthy or only slightly hyperaemic. That is, an affected but not an infected pulp.

Bonsack[30] made a plea for mummification in 1929 as a scientifically proven method, and Bossard[32] in the same year further advocated the use of Trio paste. He stated that pulp amputation is discredited because many operators use the method where it is not indicated, such as on non-vital pulps or where a condition of putrescence is present. In view of the complicated structure of canals in molars and bicuspids, total removal of the pulp and sealing of all canals is impractical, and Bossard claims that pulp amputation following devitalisation is an answer to the problem. His contention

that Gysi's Trio paste transforms the remaining pulp, rendering it an unfavourable ground for the reception of micro-organisms was supported by Muller[172] who stated that all bacteriological tests made after 1 - 14 years on residual pulp tissue showed negative results.

Castagnola and Orlay[40] reason that if the radicular pulp was not previously infected it would be kept sterile by the paste which contains a potent antiseptic.

Levine in 1934[150] reviewed the literature on pulp devitalisation and mummification and recommends this method for the treatment of fine canals in multirooted teeth, a method 'which has given others such certain success'. He states that in his experience over 20 years involving 500 cases, pulp amputation is rational, simple, and economic and when properly performed gives results unobtainable by any other method.

In 1939 Easlick[68] proposed a paraformaldehyde devitalising agent for use in children as a means of avoiding the use of local anaesthesia. Paraformaldehyde is a polymerised form of formaldehyde and is derived from saturated solutions of formalin. Following pulpotomy of pulp tissue devitalised by paraformaldehyde, Easlick placed various zinc oxide based pastes over the pulp stumps. Andrew[2] in 1955 recommended this method of devitalising teeth instead of using local anaesthesia. After 7 days the coronal pulp may be removed and mummifying paste applied. She states that root resorption takes place a little more rapidly than in vital teeth.

Trio paste was used by Inskipp[127] in 1941 in an effort to sterilise infected pulps. The paste was inserted into infected pulp chambers of 118 patients and 40 of these treated teeth were restudied one year later by clinical and radiographic methods. 27 of the 40 showed no evidence of further infectious changes, while 10 gave X-ray plates which were of doubtful interpretation. Inskipp states that since more than half the teeth with infected pulps can be saved, such treatment would seem to merit careful consideration in selected cases before extraction. This would appear to be the first recorded article describing a single stage devitalisation/mummification procedure.

Juge[130] recommends Trio paste for filling those canals which cannot be mechanically cleansed. It is hoped that the lack of mechanical preparation will be compensated for by chemical penetration. Castagnola and Orlay[41] in discussing the Walkhoff method state that in canals which cannot be opened up, Trio paste should be inserted as far as possible to take advantage of the disinfecting power of the formaldehyde.

Nacht[175] in 1956 proposed a single stage mummification technique for conserving primary teeth with a poor prognosis. In such cases where pain is present. calcium hydroxide following pulpotomy gives a high percentage of failures. Nacht recommends the use of Oxpara, a paraformaldehyde containing paste. The only criterion is vitality; pulpotomy is carried out under local anaesthesia and then Oxpara is applied to the pulp stumps. The technique was carried out on 467 teeth which were observed for 5 years. Only 30 teeth had to be extracted because of acute symptoms and the treatment therefore allowed many teeth to be retained which were otherwise doomed to extraction. There seemed to be progressive loss of bone in the bifurcation leading to early loss of deciduous molars but the treatment did not result in acute apical disturbances or fistulae as was the case with so many teeth treated with calcium hydroxide.

Davies[61] 1962, prefers a two stage procedure in the mummification of deciduous teeth. He recommends Easlick's paste for devitalisation followed by pulpotomy and covering of the pulp stumps with Gysi's Trio paste or Oxpara. He reasons that in older deciduous teeth, the blood supply appears poor and one should not expect a great deal of success from the use of pulp capping or vital pulpotomy. The mummification pastes, which are fortified with other bactericidal agents besides formaldehyde, are relied upon to sterilise the pulp remnants and to maintain sterility. Davies claimed a high level of success for this technique - 87%. He states that this technique may be used in the presence of painful symptoms. The onset of pain indicates that inflammatory changes have already taken place[192] and conservative therapy will generally prove unsuccessful. Non-vital techniques are therefore indicated.

It will be noted that paraformaldehyde is used to both devitalise and mummify the pulp. The only reason for using a two stage procedure appears to be the avoidance of the use of local anaesthetics. In cases of necrotic pulps, Davies recommends initial removal of coronal pulp remnants, followed by dressing with Oxpara liquid which contains liquid formalin. Oxpara paste is then used over the unfilled canals.

It is of interest to note that the Oxpara material was used by Strinberg[239] for teeth with non-vital pulps where the canal could not be prepared to the apex.

Very little information appears in the literature as to the effect of paraform mixtures on the vital pulp, or the reaction of the periapical tissues to paraformaldehyde pastes which have been extruded into the periapical tissues.

Orban[182,183] experimented with dogs' teeth in 1933. When paraformaldehyde was placed on the surface of the exposed pulp, necrosis occurred and inflammation of the periapical tissues resulted. At a distance, however, paraformaldehyde stimulates a defence reaction. When a mixture of 10% paraformaldehyde in zinc oxide was placed in a cavity having no exposure, the pulp remained vital and much secondary dentine was deposited - much more than when only cement or amalgam were used. In a concentration of 25% paraformaldehyde, fibrous degeneration of the pulp occurred with development of bone deposits.

Gottlieb et al[93] overfilled and underfilled dogs' teeth with zinc oxide and eugenol containing 5% paraformaldehyde. In overfilled cases, periapical abscesses were noted in some cases, but when the material was confined to the canal, normal secondary cementum covered the apex. This latter observation coincides with the experimental results of Rowe[202] who filled root canals in cats to the cemento-dentinal junction with various compounds. A mixture of 5% paraform with zinc oxide and barium sulphate or glycyrrhetinnic acid gave very favourable results, and only one tooth showed moderate periapical inflammation.

Kojima[136] compared the effect of arsenic and paraform when applied to humen pulps. He found that paraform produces changes 24

hours later although the effect is morphologically the same. Disorder and vacuolisation of the odontoblast layer appears first, hyperaemia starts next near the area of drug application, extending toward the apex, then bleeding and necrosis follows. With arsenic, bleeding and necrosis of the pulp appeared markedly in cases examined 24 - 48 hours after application of the drug, whereas with paraform paste these changes did not appear in many cases until 48 - 72 hours after application.

Inskipp[127] placed Kysi Trio paste in pulp chambers of monkeys' teeth. After 16 months the tissue was examined and in 6 out of 7 cases osteoid tissue or cementum had formed around the apices or in the canals.

Hargreaves[106] applied paraformaldehyde paste to seven deciduous teeth for 3 - 4 days then covered the pulp stumps with zinc oxide, xeroform and eugenol. The period was not sufficient for complete devitalisation and extensive necrosis was observed in only one deciduous radicular pulp. In all other cases vital radicular pulp was apparent beneath a zone of necrobiosis underneath the paste.

In 1958 Wong[253] studied the effects of a paraformaldehyde paste on the amputated pulp and periapical tissues of primary and permanent teeth in Rhesus monkeys. He found a similar inflammatory reaction in both teeth as well as degenerative changes in the bone.

<u>The Formocresol pulpotomy technique</u>

The technique for pulpotomy which uses formocresol and a zinc oxide eugenol cement has evolved, with many modifications, from one that was devised by Sweet in the early 1920's. Formocresol, originally introduced by Buckley in 1904 as a strong disinfectant for treatment of pulpless teeth, does not stimulate a healing response but rather tends to fix the pulp tissue to a variable depth depending on the length of time the formocresol is in contact with the pulp.

Foster[86] in 1936 described a successful treatment using formocresol, and Doyle et al[67] recommend formocresol containing 19% formalin for carious exposures in deciduous teeth, stating that this treatment is superior to calcium hydroxide therapy. They judged treatment with calcium hydroxide to be 50% successful on a microscopic basis whereas the formocresol group was 92% successful.

Doyle indicated that in teeth treated with formocresol, the 'fixed' area of pulpal tissue showed a transition to healthy vital tissue in the apical area.

Sweet in 1963[240] showed only 15 failures out of 561 deciduous molars which had been dressed with formocresol. This failure rate of less than 3% is remarkable, but unfortunately there are few reports available concerning treatment of adult teeth by this method.

Massler and Mansukhani[155] reported on the effect of formecresol on the pulps of 43 human primary and permanent teeth. The surface of the pulp adjacent to the formocresol became eosinophilic within a few minutes and was thought of as being a fixation of living tissue. After 7 to 14 days, three distinctive zones appeared in the radicular pulp: 1) a broad eosinophilic zone of fixation, 2) a broad pale staining zone with poor cellular definition and 3) a broad zone of inflammation diffusing apically into normal pulp tissue. After 60 days to one year the pulp was progressively fixed after the acute inflammatory phases with ultimate fibrosis of the entire pulp.

Emmerson et al[74] investigated the effects of formocresol on the pulp tissue of 20 asymptomatic humen primary teeth. The reaction of the pulps varied with the total time the formocresol was in contact with the amputated stump, the changes varying from surface fixation to a complete calcific degeneration. Following application of the drug for 5 minutes to three days, normal pulp tissue was evident below an area of fixed tissue and there was an absence of inflammatory cells. They concluded that the use of formocresol in pulp therapy may be classified as either a vital or a non-vital technic depending on the length of time of the formocresol application. They also felt that the formocresol used in this technique does not induce the same pulpal changes as paraformaldehyde compounds. After applying formocresol to normal primary pulp tissue for seven days, Dietz[63] then restored the teeth with zinc oxide and eugenol and extracted them after periods ranging from 24 hours to 16 weeks. He noted that the pulpal tissue seemed to wall off the surface necrosis by forming an 'acellular collagenous like band', and this widened as the time interval increased. Inflammation was mild.

Spadding et al[233] compared the action of formocresol therapy with calcium hydroxide in teeth of monkeys and the results give the impression that the former therapy is the superior form of treatment. One drop of formocresol was placed in contact with the pulp stump for 5 minutes then zinc oxide and eugenol plus one drop of formocresol was used as a dressing. Many teeth had pulp tissue fixed to a depth of ½ to ⅔ the canal length. Of 21 teeth, 20 contained vital tissue in the apical regions. Calcium hydroxide treatment of 25 pulps resulted in 10 necrotic pulps.

Berger[18] showed that after formocresol treatment of pulpotomised human primary molars for 5 minutes followed by sealing with zinc oxide eugenol plus formocresol for periods ranging from 3 to 38 weeks, characteristic 'fixed' tissue was seen in the coronal ⅔ of the canal. Odontoblasts and other cellular elements were well preserved. In the middle third of the root, early coagulation necrosis was seen with loss of structural integrity. In the apical third, there was absence of cellular detail except for isolated blood vessels containing decomposed blood cells. After 7 weeks he noted ingrowth of connective tissue apically replacing necrotic pulp tissue and after 35 weeks this extended close to the amputation site. It is of interest to note that control teeth treated only with zinc oxide and eugenol showed active and chronic inflammatory reactions consistently with some areas of internal resorption.

Redig[195] reported successful results using a single stage partial mummification technique. Formocresol was applied to the amputated pulp stumps for 5 minutes then zinc oxide and eugenol with one drop of formocresol was inserted as a base. This, he asserts, is as effective as a two stage procedure which leaves the initial formocresol dressing for 3 days. Beaver et al[15] as a result of histologic examination of formocresolised pulpotomies, noted that the addition of a drop of formocresol to the zinc oxide base does not appreciably alter the reaction of the underlying pulp.

This review of the literature traces the development of the formocresol pulpotomy technique to its present form. Essentially it uses formaldehyde which penetrates the superficial pulp tissue by slow

diffusion, fixing both the tissue and any micro-organisms present. The combination with tricresol reinforces the bactericidal potency of the solution. The success of the technique rests on the preservation of vital tissue in the apical third of the pulp canal.

The N_2 controversy

The rather extravagant claims made by Sargenti and Richter in their book 'Rational Root Therapy'[204] which outlines a technique for using the N_2 material, has resulted in a rejection of the method by some clinicians, but in view of the less demanding technique of use, it enjoys considerable popularity amongst others. N_2 is a paraformaldehyde containing paste and although the content is small (4.765%) the technique must be regarded as akin to a single stage mummification procedure.

It does, however, have the attraction of simplicity when dealing with vital cases. Richter[197] states that using the N_2 technique, in vital cases, preparation of the canal to the apical third is all that is required. The authors state that much less time is required for mechanical preparation of the canal, because small pulp residues in narrow or curved canals may be left in place. N_2 'immobilises' and 'permanently protects' such remnants. It is possible to cap narrow canals in the middle of the root canal or even in the neighbourhood of the crown. Fine canals which cannot be found are capped with N_2 in the pulp chamber. With deciduous teeth, only an amputation of the crown pulp is necessary, the pulp stumps being covered with N_2. This is indeed a simplification of root canal therapy.

It is difficult to believe such comments as: 'No granuloma can form on a root filled with N_2'; 'bacteriological cultures are not needed in view of the fact that the particular disinfectant qualities of N_2 make bacterial life within the root canal impossible'; 'necrotic tissue which cannot be removed is neutralised by the permanent N_2 filling'. The authors also talk about the 'far reaching effect' of N_2 enabling the material which partially fills an infected canal to effect healing of apical areas.

The actual effect of the material on the tissues is difficult to-

to discern, although the authors claim that vitality of the apical region is maintained under the root filling. Richter[197] states that the tissue in direct contact with the N_2 mixture slowly transforms into a kind of fibrillar connective tissue forming a thin membrane between the N_2 deposit and the pulp remnant. 'Eventually the actual border becomes as structureless as scar tissue. All this goes on by a slow metaplasia and nowhere does any devitalisation or necrosis of tissue result'.

However, in their book, they state that some necrosis of the superficial layers of tissue in contact with the paste occurs due to the paraformaldehyde, thus forming a sclerotic membrane delimiting the vital tissue. 'The favourable results obtained by calcium hydroxide for the capping of vital exposed pulps are due to the superficial necrotisation of the pulp tissue. In other words, it is necessary that an insulating membrane be formed between the living tissues and the foreign body'.

The usefulness of having paraformaldehyde in a root filling paste is supported by some research workers. Hyakusoko[124] reported on the efficiency of several types of root filling paste after studying the apex and periapical tissues of several teeth gained by an apicoectomy approach. He stated that calcium hydroxide and a paraformaldehyde containing cement induced the best healing. Chloropercha was much inferior and zinc oxide and eugenol gave the most unfavourable results.

Kuroiwa[142] reported that N_2 produced good results in root canal therapy but only in uninfected teeth, stating that the material was not effective in treatment of infected canals. The work was carried out in dogs, and in some cases paste was deliberately extruded into the periapex. Encapsulation of this paste was described as extremely poor. Sargenti and Richter warn against the extrusion of pastes into the periapical tissues because it is non resorbable. However, they state that N_2 heals into the periapical tissues without complication. This is not verified by Kuroiwa.

Overdiek[188] however, found that N_2 was well tolerated in guinea-pig skin and muscle and was walled off by a connective tissue capsule after 9 weeks.

The Council on Dental Therapeutics does not support the N_2 method[50]. They point out that periodically there have been waves of interest in root canal filling pastes containing formaldehyde for use as a means of 'sterilising' and filling a root canal in one treatment. No adequate evidence is available however to indicate that a single treatment filling technique using such a paste will result in sterility in any large percentage of cases. The Council does not consider the antibacterial properties of N_2 are any greater than camphorated parachlorphenol, and they state that the material would be extremely irritant when extruded into the periapical tissues. The intentional underfilling of a canal is questioned and they draw attention to the fact that Ingle[125] reported 75% of root canal therapy failures were associated with failure to obliterate the root canal. In fairness to Sargenti and Richter, however, it must be pointed out that a technique is presented for cutting the pulp tissue in the apical third of the canal. If this remnant remained vital, the periapex would remain intact.

Rowe[200] considers that the N_2 technique is a useful aid for conserving vital exposed posterior teeth where conventional treatment would be difficult. He followed 90 cases for 6 months to 3 years and reported only one failure. He examined some teeth histologically after the filling had been in place for up to 2 years and reported vital tissue extending beyond the N_2 root filling and considerable deposits of calcified tissue were observed in the apical part of the canal. He states that animal experiments have confirmed the tolerance of pulp tissue to N_2, and reports that 9 out of 12 cat root canals filled with N_2 did not show any adverse reaction around the apices of the teeth[202]

In a further communication Rowe[201] describes three cases examined histologically. In one case of partial root filling with N_2 there was a zone adjacent to the N_2 containing pulp debris and tissue fixed by the N_2 corresponding to the sclerotic zone described by Sargenti and Richter. Under this sclerotic zone the pulp tissue was normal and a hard tissue barrier was being formed. In two other cases similar results were observed with a well defined partly calcified sclerotic zone beneath which normal pulp was preserved. Much cementum was deposited apically. He concluded that N_2 is well tolerated by pulp

remnants; the only damage is next to the paste. He points out that the 4.7% paraform content is below the limit set by Orban in 1936 who advocated a 5% concentration with zinc oxide and eugenol in periodontal treatment. The action of the paraform is transient and can only act during the setting of the material. Ostby[186] states that 'it has been established that a temporary chemical irritation does not jeopardise the healing of the periapical tissue'.

Clinicians are divided in their views. Curson[54] comments on the fact that although the N_2 material is presented as a bland medicament which is 'biological' in its effect, it is in fact irritant. He considers that the action of N_2 is to form a barrier of necrosis on the surface of the amputated pulp and that the final outcome is probably a slow degenerative change in the remaining pulp tissue producing a result very similar to that of mummification.

Curson[55] states that there is little agreement between reports on the clinical use of N_2 and considers that much further study is required. He quotes the following figures from European literature:-

Author	No. of teeth	Observation period	Failures
Barman & Eberaberg 1955	2000	3½ years	1 = 0.05%
Tachamar 1963	14	-	10 = 71%
Tolander 1966	165	5 - 7 years	13 = 8%

Hampson[105] feels that, despite the adverse report of the Council on Dental Therapeutics, the N_2 method of treating exposed posterior pulps promises much.

Crabb[52] considers that more evidence is needed about the direct action of paraform on pulp tissue. He feels that N_2 sealer probably acts much in the same way as calcium hydroxide producing a superficial necrosis and permitting rather than stimulating repair.

Nicholls[176] from his own work suggests that the response of residual vital pulp to N_2 may vary according to the level to which the root canal is filled. Where this is within a short distance of the apical foramen, a favourable result in the form of fibrosis is possible. Where the pulp is severed well short of the apical foramen

however, devitalisation and necrosis of much of the remaining pulp may occur. He regards the placing of short root fillings as 'doubtful'.

Silbert[228] and Ehrmann[69] condemn the N_2 technique and state that it contravenes many of the basic teachings of endodontic practice. Ehrmann cites a case in which N_2 extruded into the mandibular canal of a patient gave rise to persistent pain and paraesthesia. In a further report, Ehrmann[70] mentions three cases of severe pain following overfilling, one involving the antrum. Ostrander[187] comments on the case report of Ehrmann's and points out that all new therapeutic agents which are introduced with exaggerated claims of effectiveness and of good results obtainable with greatly simplified and shortened techniques as compared to conventional treatment practices, should undergo a period of careful study and thorough evaluation before they are used widely in the profession.

In reply to Ehrmann's paper, Sargenti[203] reaffirms that gangrenous teeth require several sittings for cleansing and filling to the apex. He quotes Coolidge and Kessel, Textbook of Endodontology (1956)[48] as saying that 'a satisfactory filling in a root canal from which a vital pulp has been removed is one that obliterates the canal as far as the pulp has been removed. If the pulp has been destroyed and removed to the apical foramen, the filling must be extended to the apical foramen, but if there is a small amount of living tissue near the apical foramen that has not been destroyed or too seriously injured by mechanical, chemical or bacterial irritants, the filling should be inserted to the level of the living tissue, which will heal and remain in a healthy condition. In curved or obstructed canals, the vital pulp may be severed at the curve or at the point of obstruction. The filling must then be inserted to that level'.

Sargenti also refers to Strindberg's work wherein he claims that better results can be obtained if the canal in vital teeth is not filled exactly to the apex. Ostby[186] states that the apical part of the pulp consists of fibrous connective tissue which retains its structure and sterility even in cases of severe chronic inflammation of the pulp. Consequently the so called partial pulp

extirpation, i.e., the placing of the extirpation wound in the apical part of the canal, is the safest therapy in these cases'.

In support of Sargenti and Richter, Fish may be cited[85]. He states that 'provided that no organisms have been carried into the apical canals, it makes little difference whether the canals are well filled or not provided they are effectively sealed off from the mouth ... A sound cement filling of the whole canal is valuable. Nevertheless, root canals being sometimes tortuous, it is more important to fill the cervical $\frac{2}{3}$ expeditiously than to risk carrying infection into the apices by prolonged instrumentation'. Thus Fish agrees with Sargenti and Richter in advocating prompt closure by immediate root filling to the apical third of the canal in vital cases.

Gutteso[104] agrees with the Council on Dental Therapeutics that N_2 is a severe irritant for he found it produced ulceration in the subcutaneous connective tissue of rats after 16 days. In their book, Sargenti and Richter state that N_2 does not cause any reaction when implanted in rat muscle or in the subcutaneous connective tissue of a rabbit's ear. However, they used only traces of N_2 in their implants because as they admit 'too much N_2 would result in a foreign body irritation'.

Similar results were reported by Rapapport[194] who found that N_2 caused severe inflammation and necrosis in rat subcutaneous tissue. Feldman et al[82] compared the reaction to implants of N_2 and silver in the cortex of rabbits' jaws, and could not substantiate the findings of Rapapport. They found a connective tissue capsule around the N_2 implant which was twice to six times thicker than that surrounding the silver implant on the other side of the jaw. Capsule thickness may be regarded as a reflection on the degree of irritation and N_2 is therefore more irritant than silver, but in no instance was there tissue necrosis. Macrophages and giant cells were present around all implants, but more numerous around the N_2. More dense infiltration by lymphocyte and plasma cells was also noted around N_2 and while direct apposition of bone was commonly seen against the silver implants, this was very rare with N_2.

Snyder[232] used the root canals of dogs' teeth, both infected and vital, for the comparison of N_2 with a more standardised treatment using silver cones and silver containing zinc oxide and eugenol cement. After 6 months the periapical tissues were examined histologically.

In infected cases the periapical tissues of teeth treated with N_2 were less inflamed than the tissues around teeth filled with silver cones and cement. N_2 was found to be less irritating than the silver containing cement. Following partial pulpectomy, the apical pulp tissue beneath the N_2 became necrotic in each case and chronic periapical inflammation resulted. (It must be pointed out that attempts at partial pulpectomy in dogs' teeth may well have caused severance of the entire pulp from the apical tissues Attempts by the present author to cut the apical $\frac{1}{3}$ of the pulp from the remainder resulted in complete dislodgement of the pulp in every case).

The effect of overfilling with N_2 was studied in one case. A small area of the paste was well tolerated and walled off by cementum like tissue. N_2 appeared to stimulate the deposition of collagen and/or osteoid in large amounts at a distance. This contradicts many reports that N_2 is severely irritating t: the periapical tissues.

Experiment 49. Figs. 200 - 203, page 192.

Mesial and distal canals of the lower left 3rd premolar (s, fig. 200) were penetrated for a short distance with a bur. In the mesial canal N_2 was inserted, while distally Oxpara paste was applied to the pulp.

After 7 months, an area of rarefaction had appeared over the distal root, while the mesial periapex appeared unaffected. This is seen in Fig. 201 where an area of subacute inflammation circumscribes the distal apex, while the mesial periapex shows no cellular disturbance. In each canal the pulp tissue had been reduced to dense fibrous strands (figs. 202, 203) presumably by the action of the paraformaldehyde in the pastes. Apparently, diffusion of paraformaldehyde vapour or irritant substances in solution did not occur to the same extent from the N_2 as from the Oxpara and the necrotising action of the N_2 was confined to the pulp canal in this instance.

Experiment 50. Figs. 204 - 207, page 193.

Mesial and distal canals of the lower right 3rd premolar (s, figs. 204, 205) were entered and N_2 was inserted mesially while Oxpara was placed over the distal pulp. The animal was sacrificed 9 months later.

The radiograph taken at the time of sacrifice showed an interesting phenomenon (fig. 205). The outline of the distal canal appeared identical on both postoperative and post-sacrific radiographs but the mesial canal seen in Fig. 205 had undergone some stenosis and no longer possessed the clearly defined radiolucent lumen seen in Fig. 204. This narrowing of the canal and change in radiodensity was also seen in the distal root canal of the lower right 2nd premolar which had also been treated with N_2.

Sections showed a layer of atubular irregular dentine lining the root canal beneath the N_2 dressing (figs. 206, 207) and this must have been of sufficient density partially to obliterate the root canal on the radiograph. The pulp was reduced to a fibrous remnant. Very little reaction was apparent around the apex of the mesial root, while distally there was a moderate to severe response periapically (fig. 206) caused by the Oxpara.

Experiment 51. Figs. 208, 209, page 195.

N_2 was inserted into the distal canal of the lower left second premolar (a, fig. 208) as a pulp capping and left for 5 months. The periapical area appeared normal on the post-sacrifice radiograph and this was confirmed on examination of histologic sections (fig. 209). Pulp tissue in the canal had been reduced to fibrous strands.

Experiment 52. Figs. 210, 211, page 195.

Pulp exposure of the mesial canal had been covered with Oxpara while N_2 was used to cover the exposed pulp in the distal canal.

The effect of the pastes on the periapical tissues after 5 months was observed in sections. Mesially the Oxpara had caused degeneration of the pulp and slight periapical inflammation had supervened (fig. 210). Distally, N_2 also caused pulp necrosis. Around the apex, bundles of dense fibrous tissue had formed and these were lightly infiltrated with inflammatory cells which were predominately lymphocytes and plasma cells (fig. 211).

Experiment 53. Figs. 212 -214, page 196.

Gysi Trio paste was inserted into the coronal pulp of the lower left 2nd premolar (fig. 212). After 14 months, the radiograph showed that a periapical radiolucent area had developed. A photomicrograph of the apical region revealed bone resorption and replacement with hyperaemic granulation tissue infiltrated with round cells (fig. 213). There was a sprinkling of polymorphonuclear leucocytes in this area and some of these were present in the apical ramifications. Pulp tissue had been completely lost from the canals, and an interesting observation was the presence of some of the paste in the apical ramifications (fig. 214, taken at position 'p' in fig. 213). This material does not harden and it is possible that some of the paste had been carried apically by fluid in the pulp canals. The proximity of this paste to the apical region may account, at least in part, for the continued apical irritation.

Experiment 54. Figs. 215 - 218, page 198.

In the lower left 3rd premolar (a, fig. 215) Gysi Trio paste had been placed over a pulp exposure mesially while N_2 was inserted into a

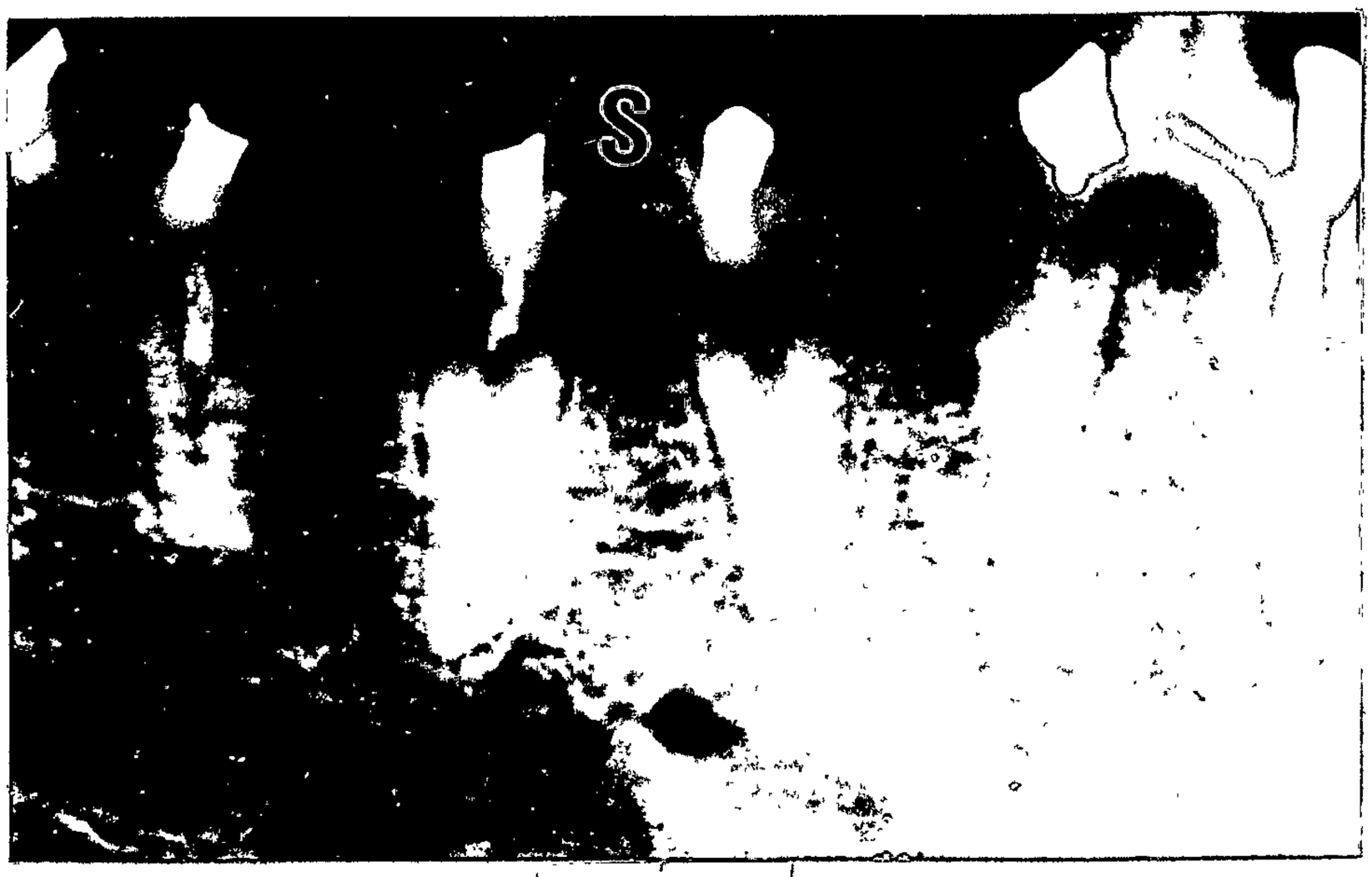

Fig.200

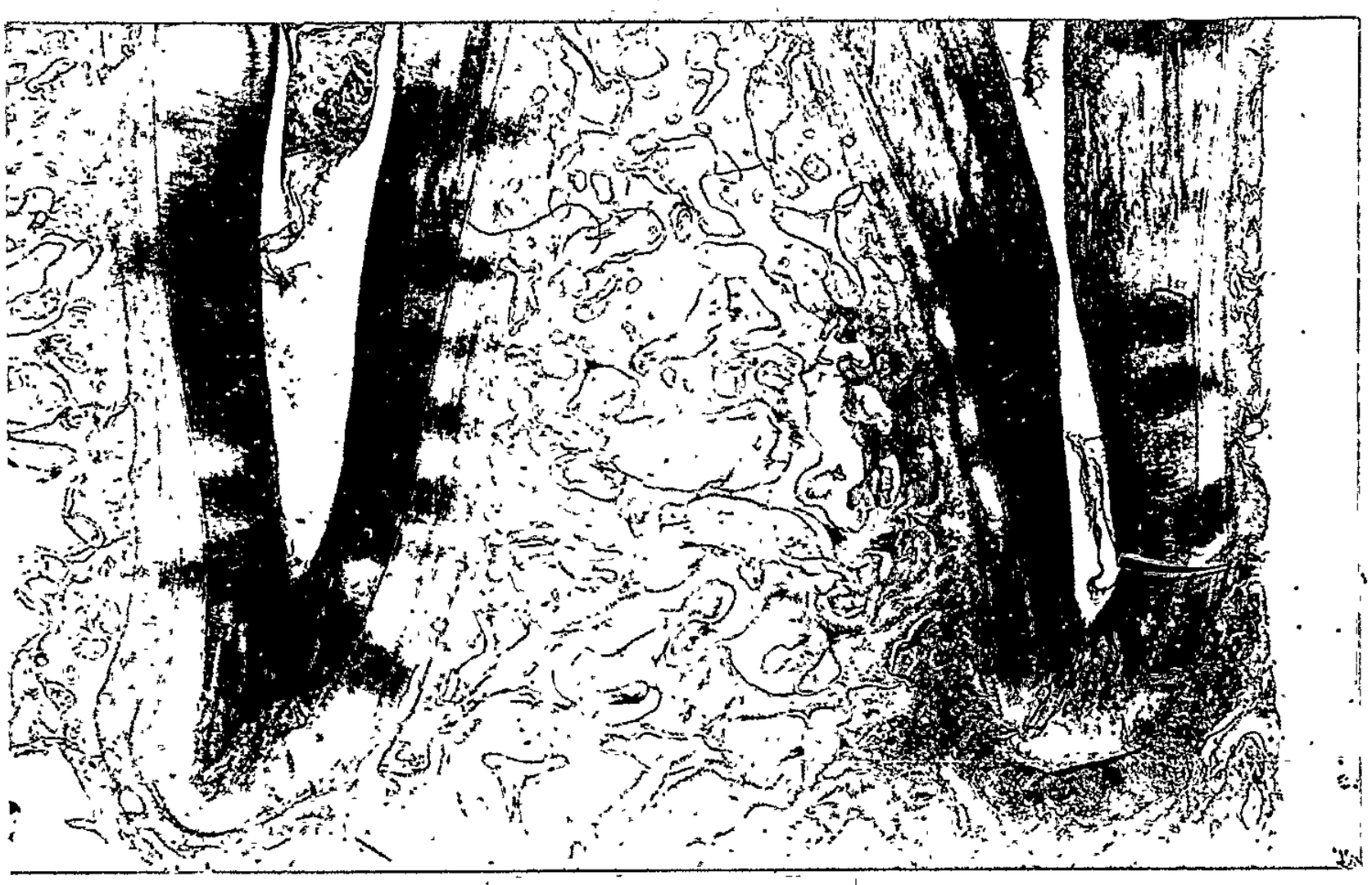

Fig.201 x10

Fig.202 x32
mesial N2

Fig.203 x32
distal
oxpara

Fig.204

Fig.205

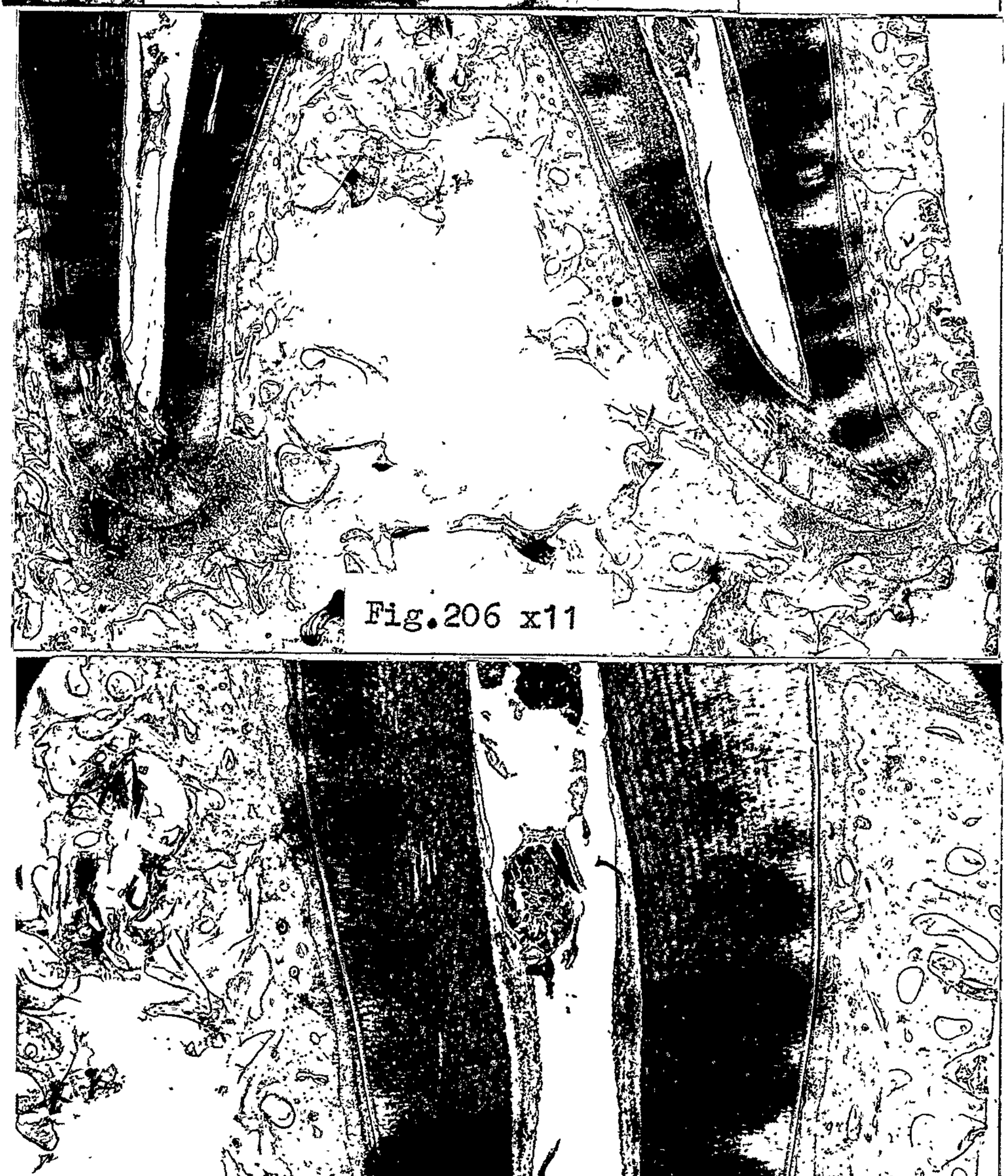

Fig.206 x11

Fig.207 x20

deep pulpotomy wound distally. The animal was sacrificed 12 months postoperatively.

The radiograph taken at the time of jaw sectioning (fig. 215) showed a large area of rarefaction over the mesial apex, while the distal periapical region appeared normal. On histologic section (fig. 216) the area around the mesial root was seen to be very vascular; bone resorption had occurred over a considerable area and inflammatory cells were scattered throughout. The pulp had been completely lost and some resorption of the apical cementum had occurred.

Distally, the pulp tissue under the N_2 had been reduced to fibrous strands, and while there was no bone resorption apically (fig. 217) there was light infiltration of inflammatory cells (fig. 218).

In this experiment N_2 did not cause the marked inflammatory changes in the periapex as seen with Gysi Trio paste.

Experiment 55. Figs. 219 - 223, page 199.

Deep pulpotomies were carried out in each root canal of the lower right second premolar. Gysi Trio paste was inserted distally, while N_2 was applied to the mesial pulp. After 12 months, each root apex showed an area of reaction.

Distally the Gysi paste had caused pulp necrosis and marked bone resorption had occurred apically (figs. 219, 220). Dense aggregations of polymorphonuclear leucocytes were located throughout the apical area (fig. 221).

Mesially the N_2, presumably by its irritant effect, had given rise to a granuloma which showed proliferation of epithelium. These epithelial strands enclosed masses of polymorphs (figs. 219,222,223).

This was the only instance of epithelial proliferation observed in dog granulomata in which there was a tendency to radicular cyst formation.

Experiment 56. Figs. 224 - 230, page 200, 201.

For a period of two weeks, N_2 had been placed over a pulp exposure mesially (fig. 224) and Gysi Trio paste had been applied to the distal pulp (fig. 227).

Beneath the N_2 there was a very small zone of pulp fixation. Below this there was moderate infiltration of inflammatory cells, many

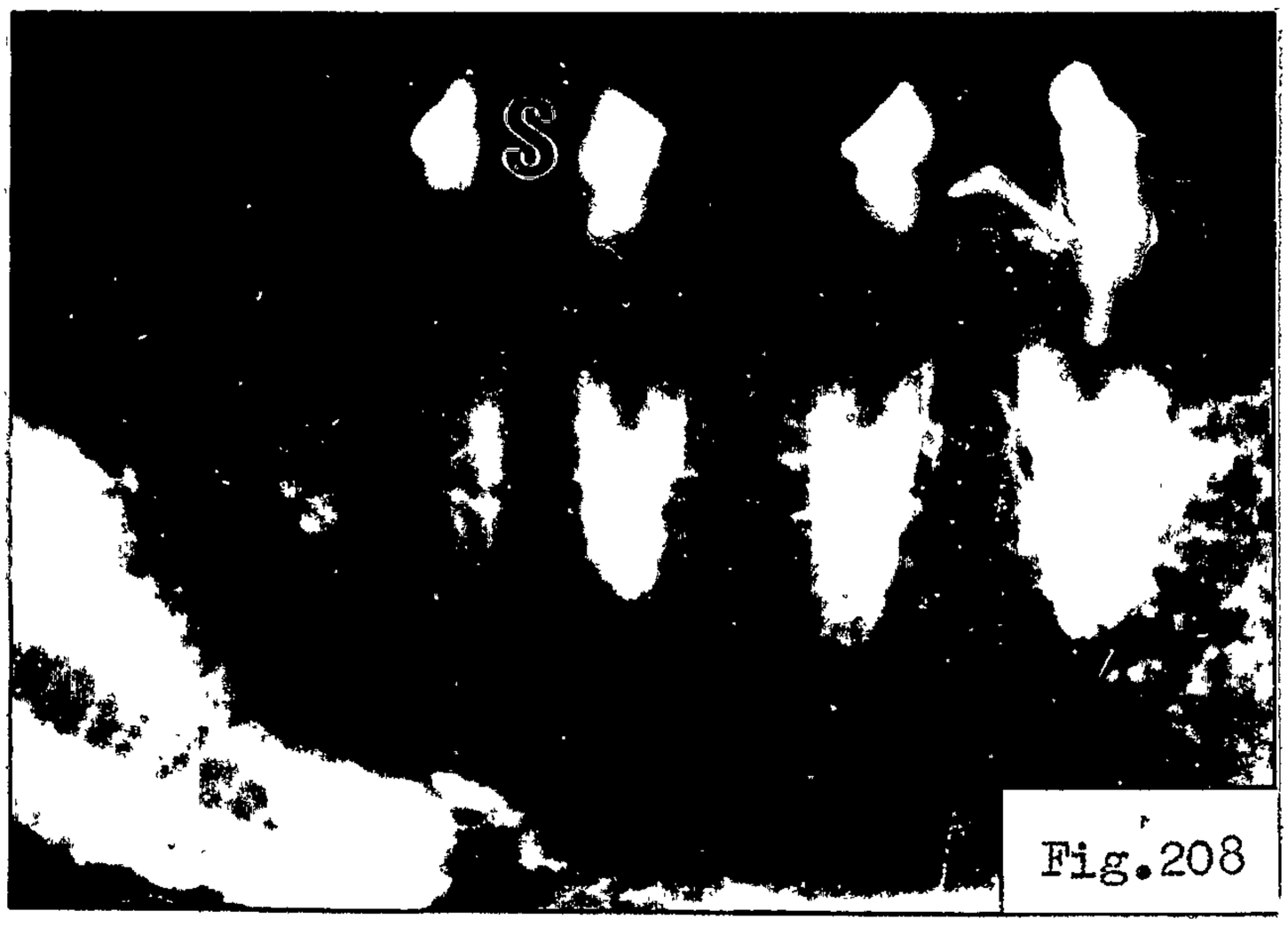

Fig.208

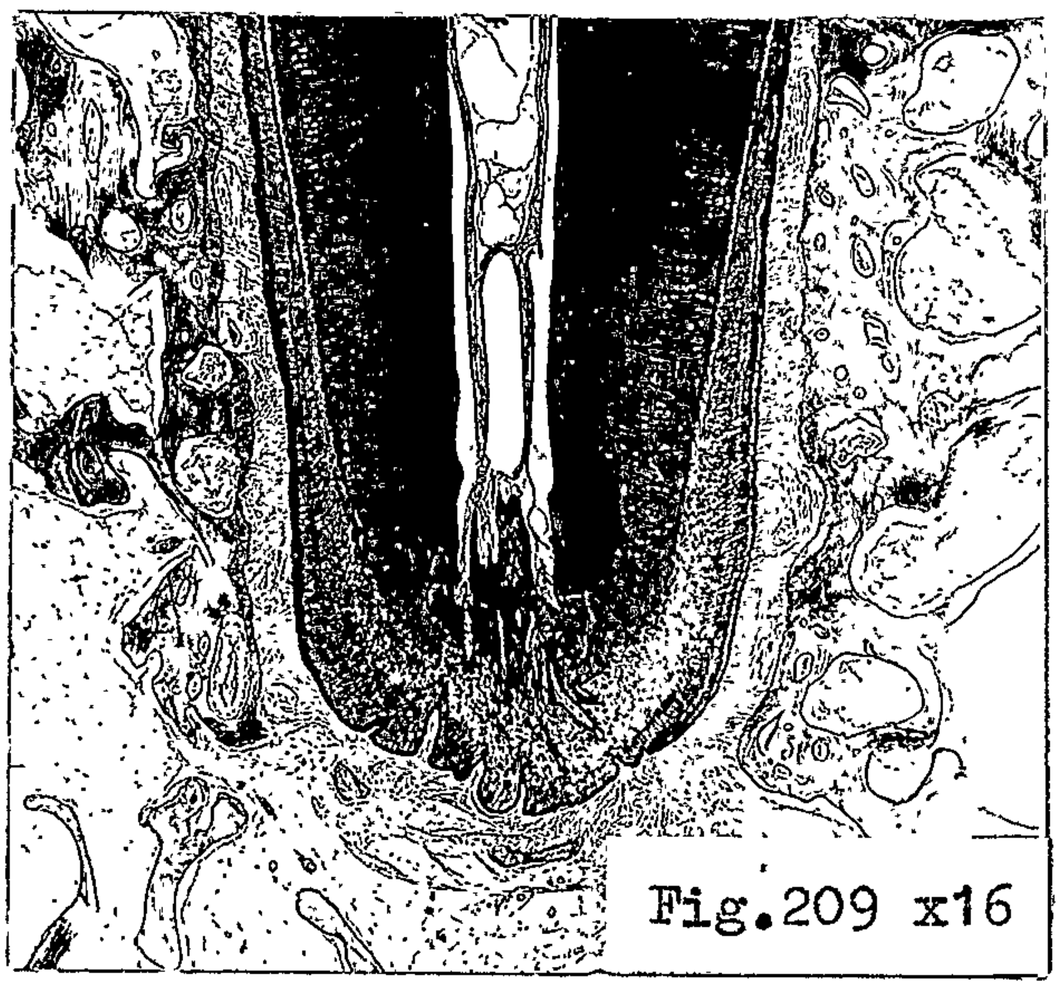

Fig.209 x16

Fig.210 x30
mesial
oxpara

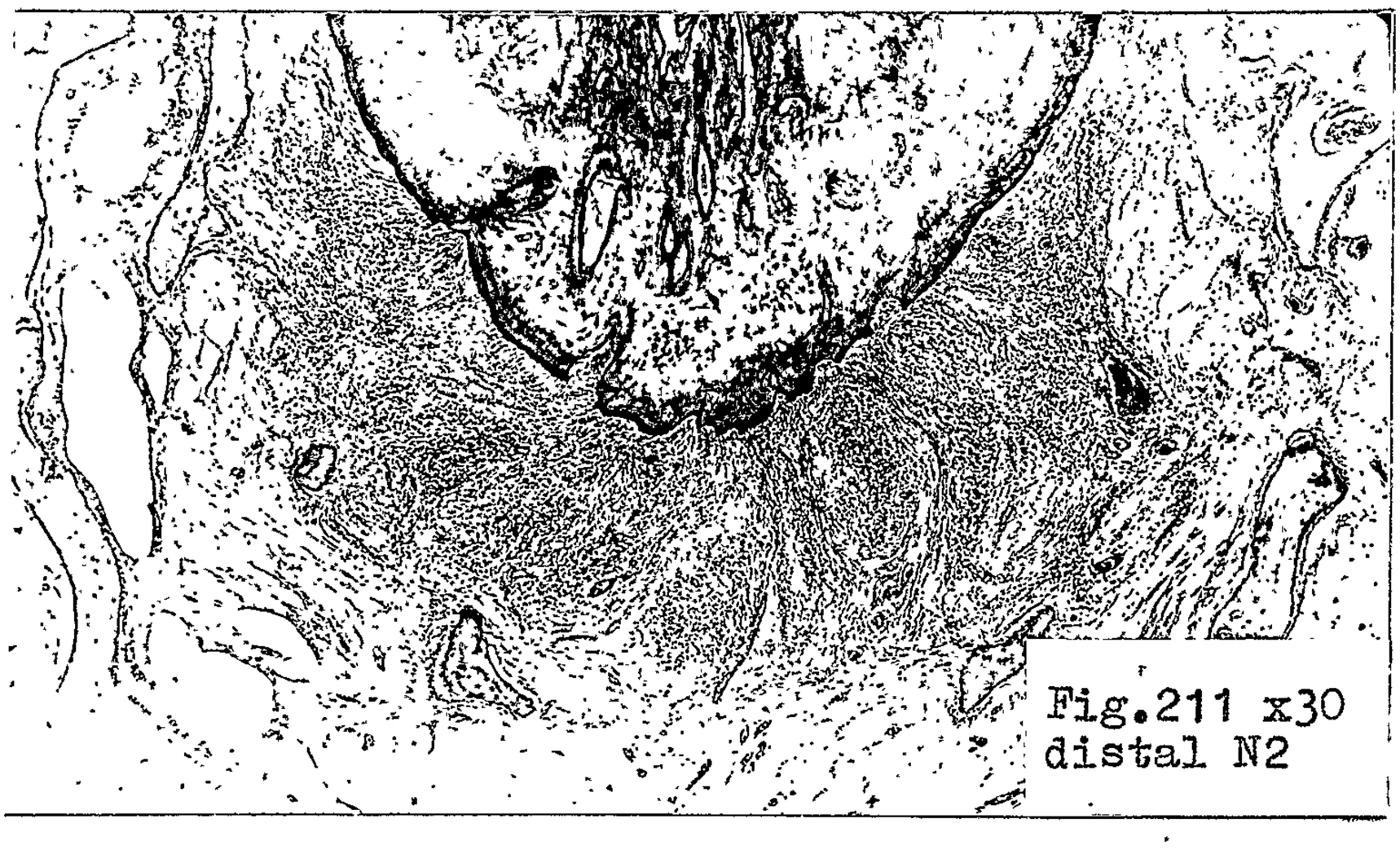

Fig.211 x30
distal N2

Fig.212

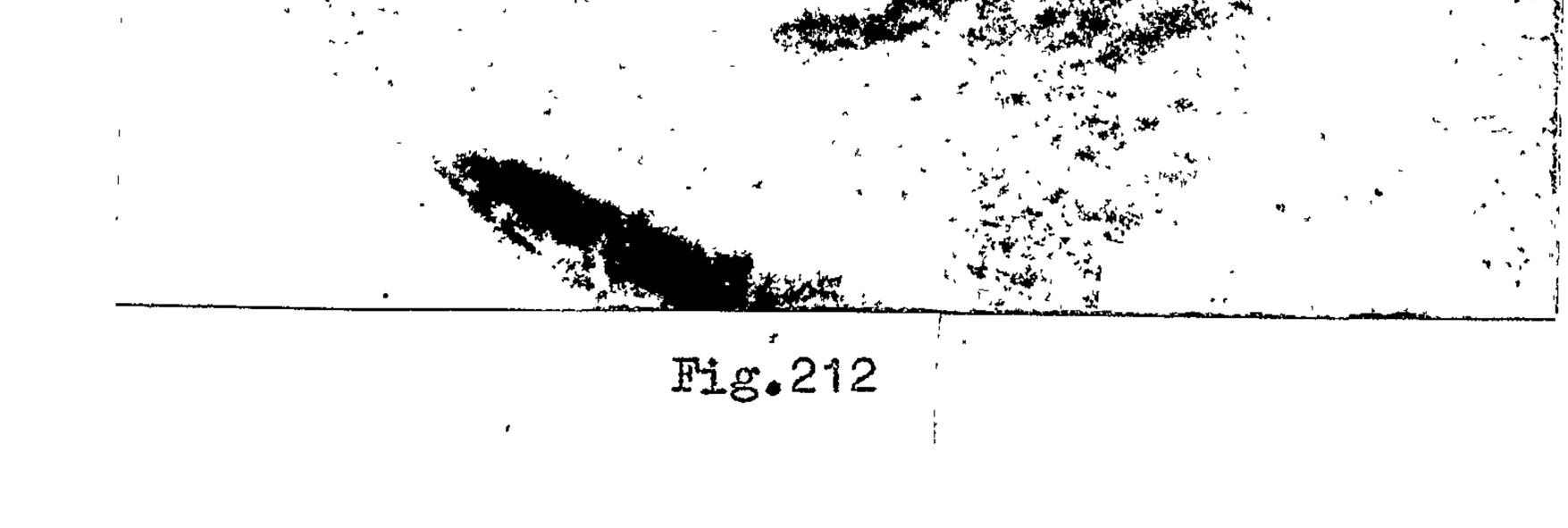

Fig.213 x20

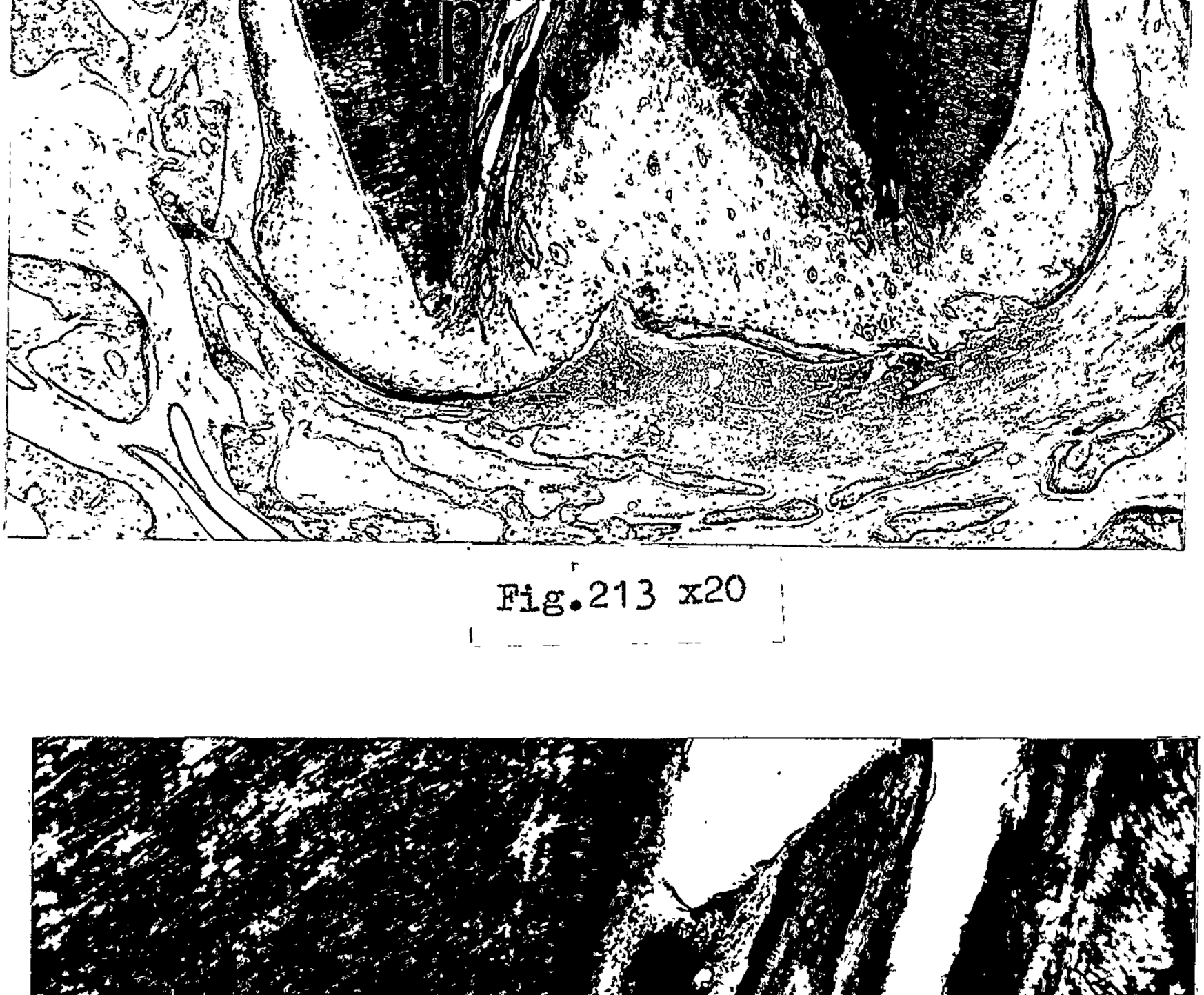

Fig.214 x125

of which exhibited pyknotic nuclear change and the pulp tissue was oedematous (fig. 225). Further apically the pulp showed some abnormalities in the form of distended blood vessels and loss of the odontoblast layer (fig. 226).

By comparison, the necrotising effect of Gysi paste was marked. Beneath the paste, an extensive zone of 'fixed' pulp tissue was observed (figs. 227, 228). The odontoblast layer was intact for a distance of approximately 4 mm, and then there was a sharp transition to oedematous degenerating tissue (fig. 229). The retention of the odontoblasts beneath the paste and their loss in the apical portion of the canal indicated that their 'fixation' was due to the formaldehyde component of the paste prior to sacrifice and not due to perfusion. The layer of degenerate tissue and necrotic inflammatory cells deep to the 'fixed' tissue was a transitional zone situated above hyperaemic tissue in the apical portion of the canal (fig. 230).

In view of the long term results with Gysi Trio paste when used on dog pulp, that is, complete loss of pulp tissue, the zone of tissue fixation must progress to complete heterolytic softening in time.

Experiment 57. Figs. 231 - 237, page 202, 203.

Zinc oxide and eugenol was applied to a pulp exposure mesially (fig. 231) while N_2 was placed over a distal pulp exposure (fig. 235). The animal was sacrificed two weeks later.

Beneath the zinc oxide and eugenol paste, blood vessels were dilated and inflammatory cells had infiltrated the coronal area (figs. 231, 232). The odontoblast layer had been lost throughout the pulp and there was generally a disappearance of normal pulp structure. Half way down the canal was an area of haemorrhage (fig. 233) and a high power photomicrograph of this region showed blood cells lying free in the tissue (fig. 234).

Distally the pulp appeared very oedematous and was reduced to strands beneath the N_2 (fig. 235). Immediately beneath the exposure was some fibrous tissue condensation, deep to which was an area of cells with pyknotic nuclei (fig. 236). There was complete loss of

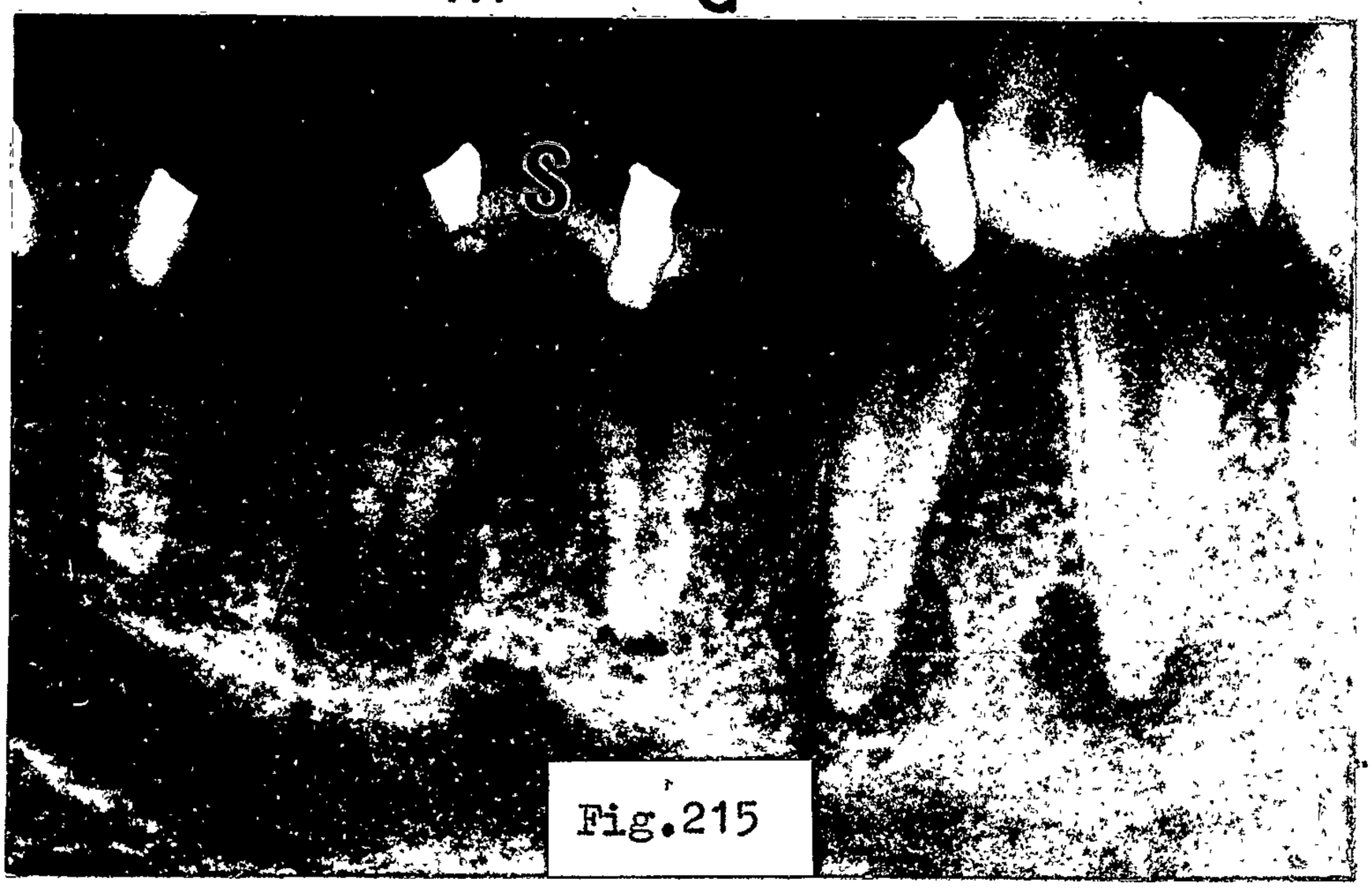

Fig.215

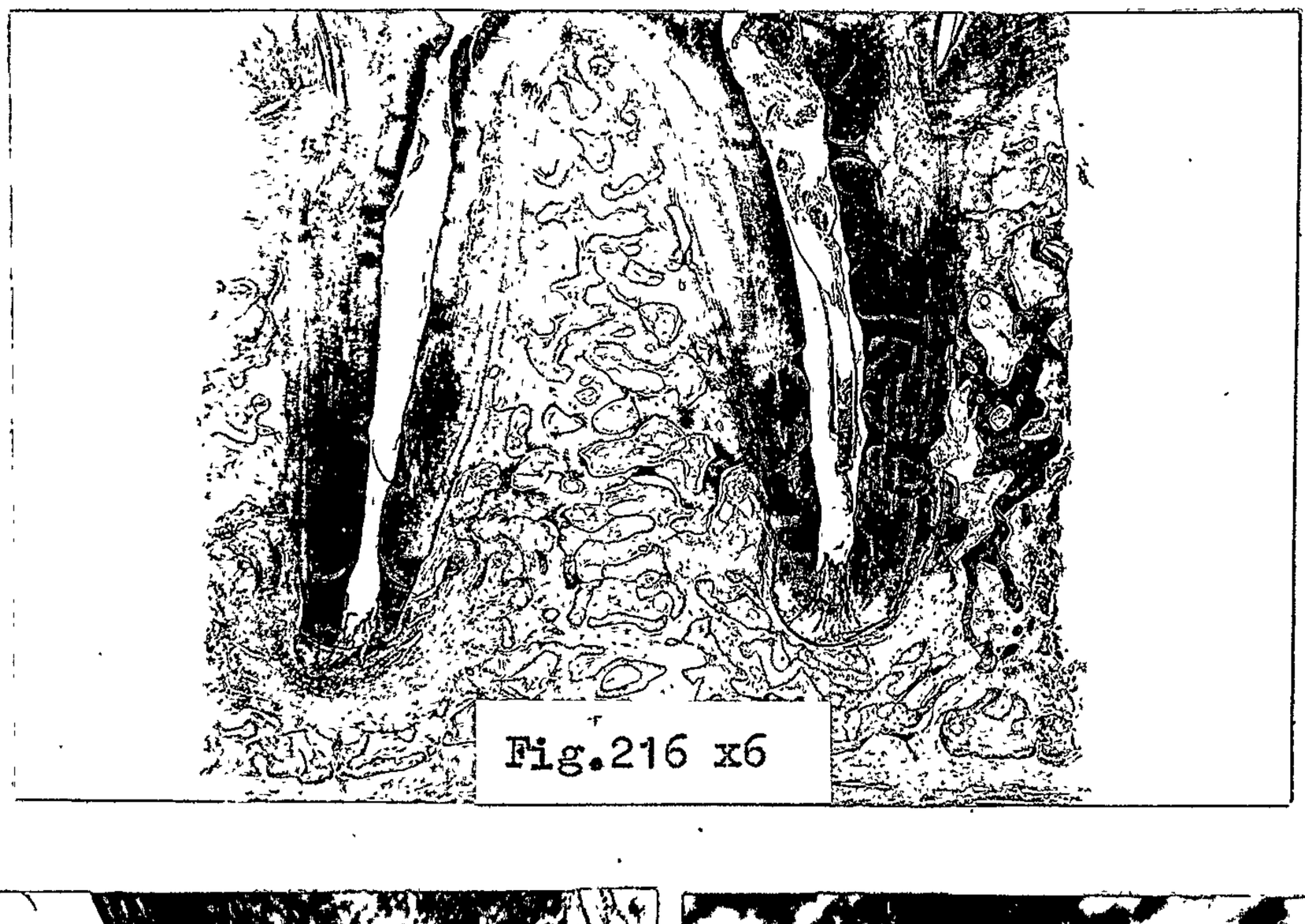

Fig.216 x6

Fig.217 x30
N2

Fig.218 x560
N2

Fig.219 x10

Fig.220 x32
Dist. Gysi

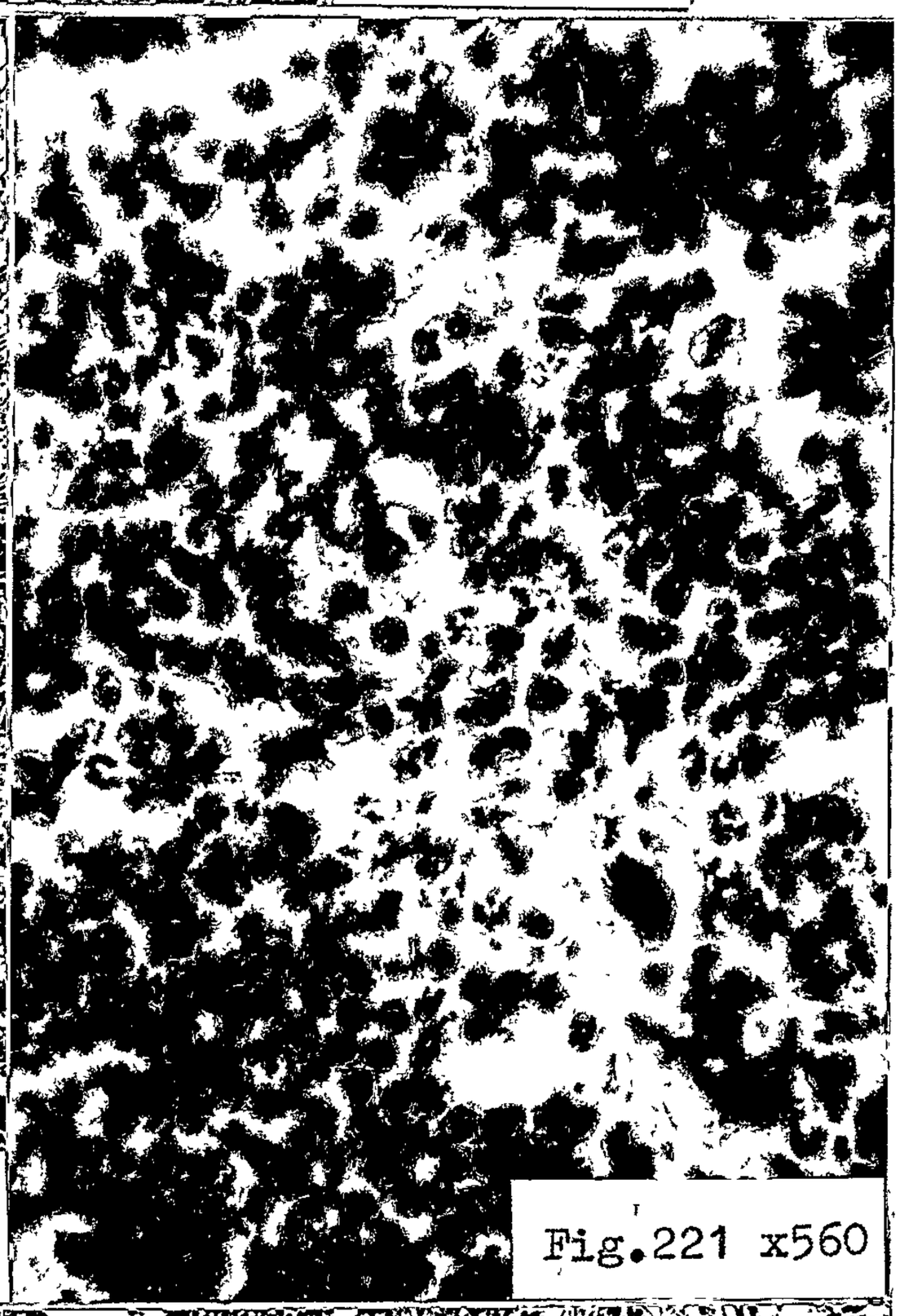

Fig.221 x560

Fig.222 x32
mesial N2

Fig.224 x20

Fig.225 x125

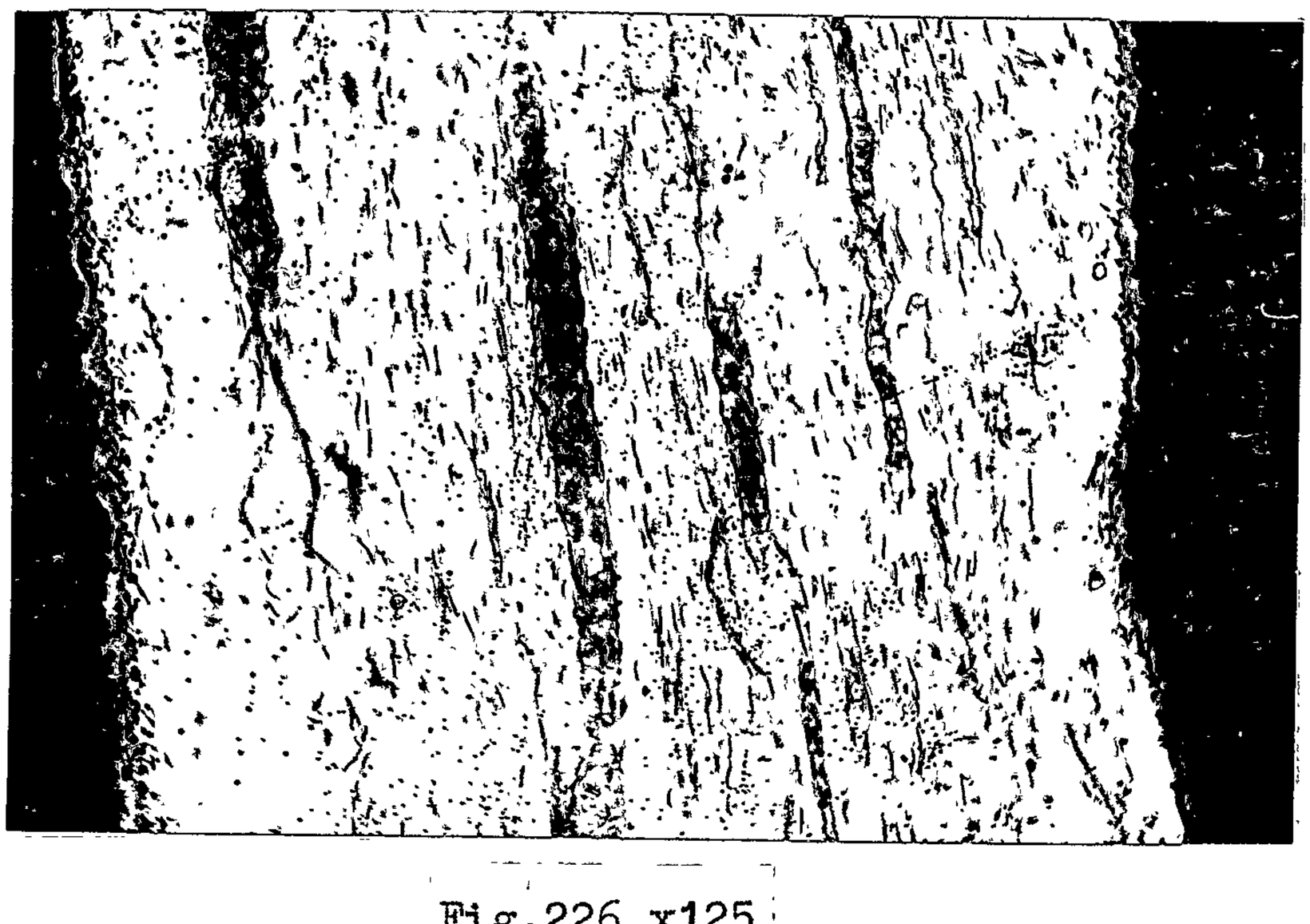

Fig.226 x125

Fig.227 x20

Fig.228 x125

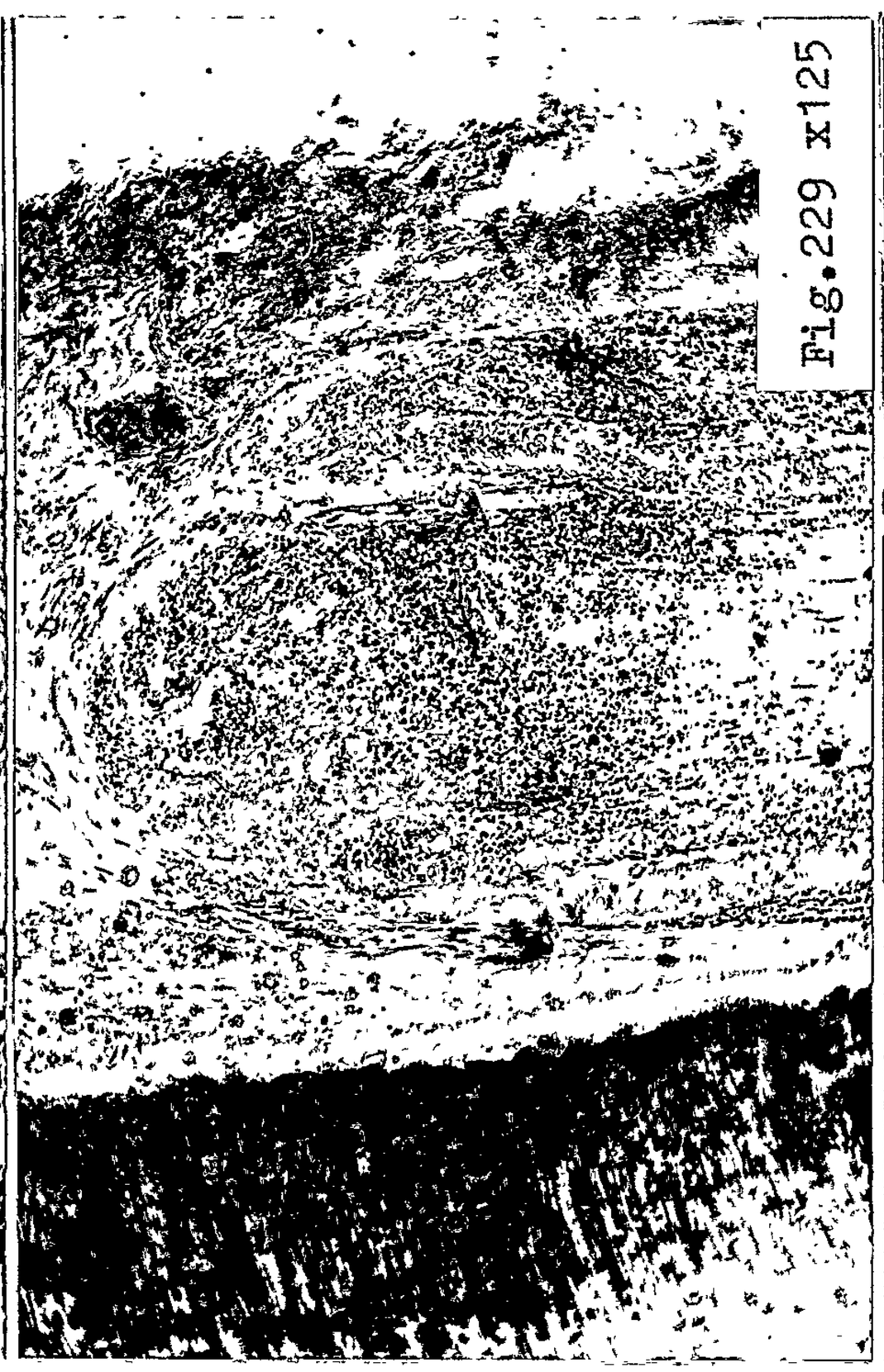

Fig.229 x125

Fig.230 x44

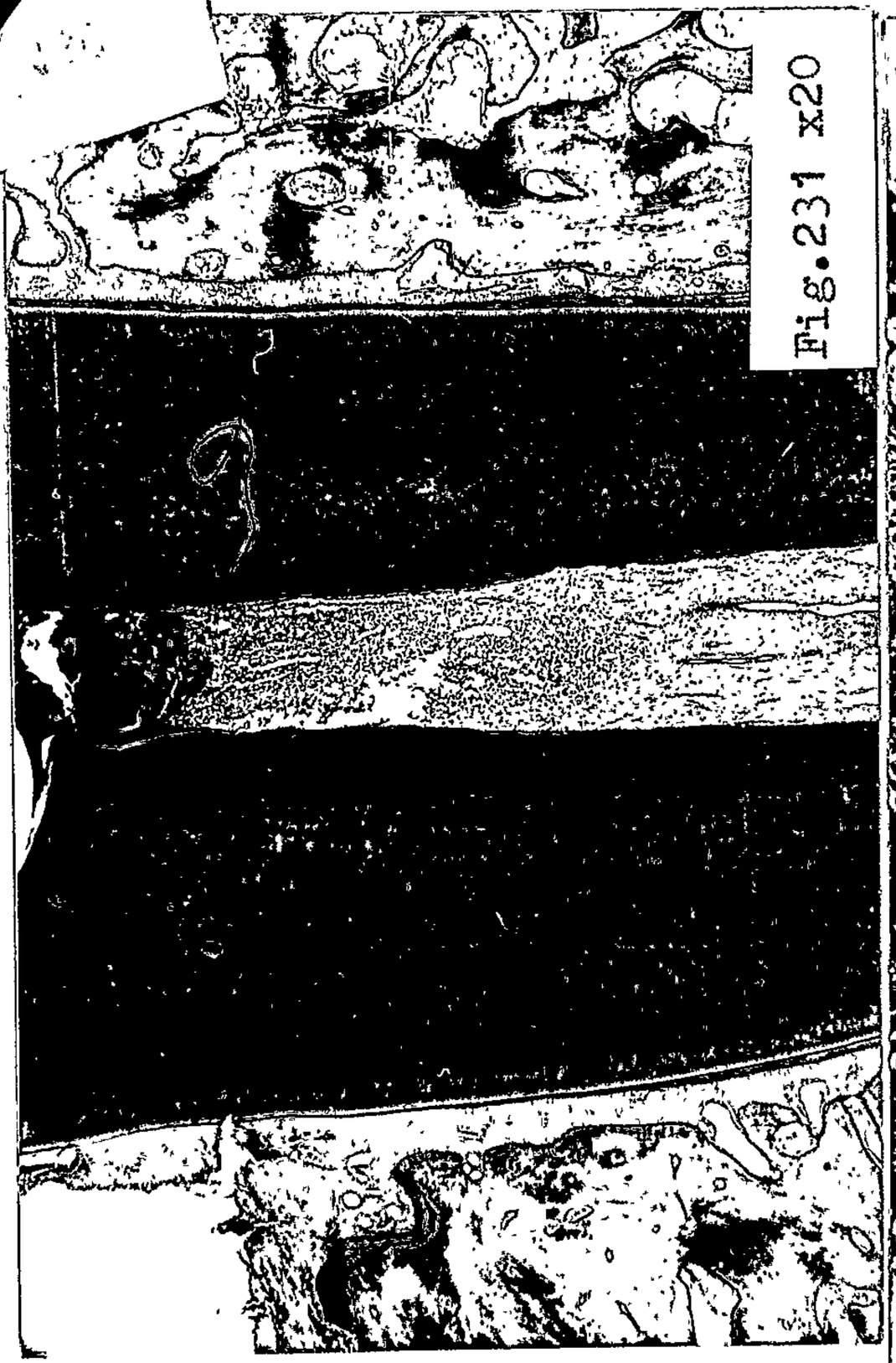

Fig.231 x20

Fig.232 x125

Fig.233 x20

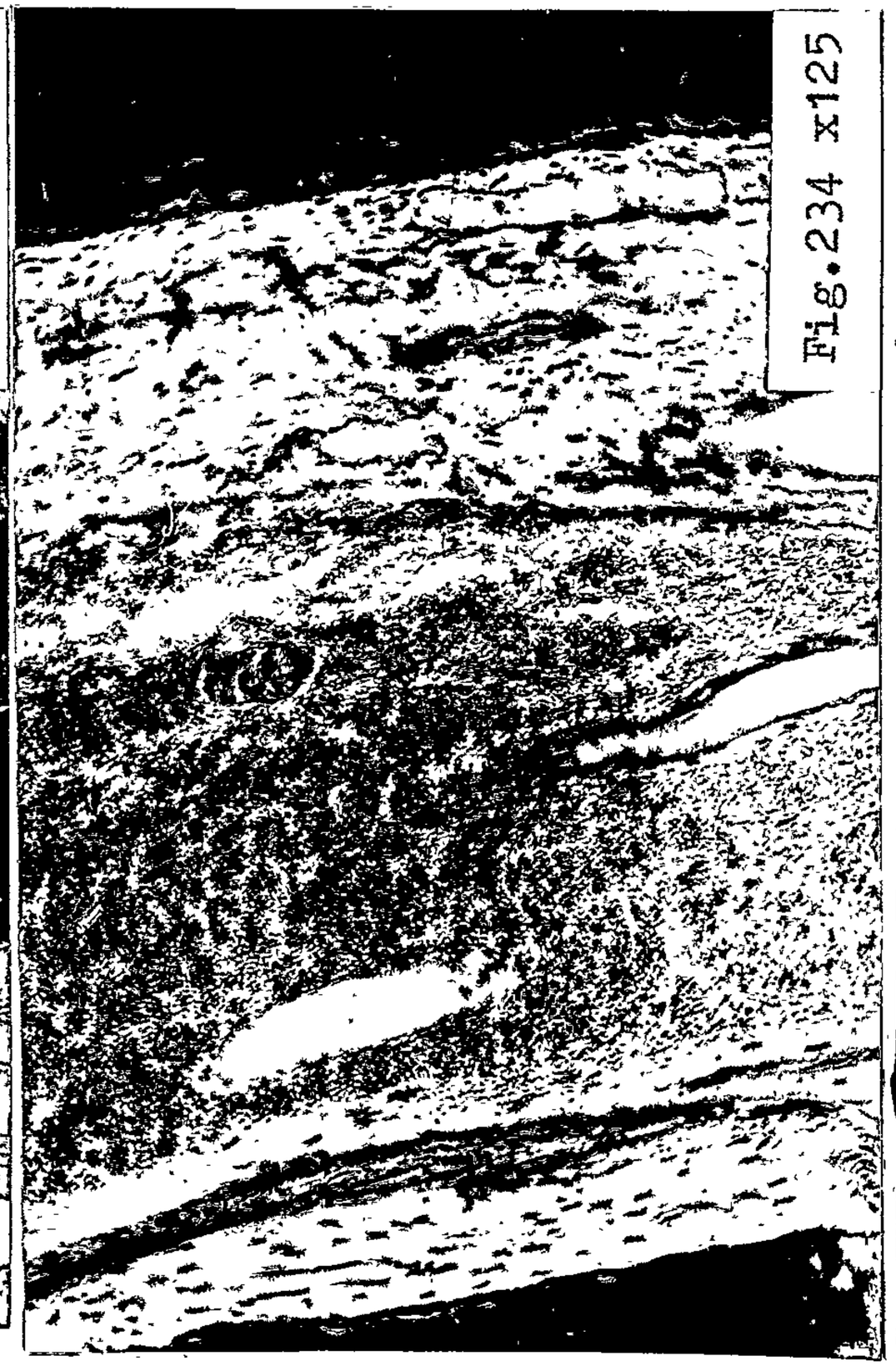

Fig.234 x125

Fig.235 x20

Fig.236 x125

Fig.237 x20

odontoblasts and the pulp appeared disorganised throughout its entire length (fig. 237). It is possible that this picture of pulp degeneration may have been partly due to inadequate fixation.

Experiment 58. Figs. 238, 239, page 205.

Six months after capping the exposed pulp with N_2, the tissue appeared to have remained vital throughout most of its extent, although the area beneath the exposure was reduced to oedematous strands.

Vacuoles had appeared in the coronal pulp, but beyond this the odontoblast layer was traceable (fig. 238). Dentine chips were encased in hard tissue coronal to the exposure, and just beneath the capping material, cells were seen trapped in newly formed hard tissue lining the canal wall (fig. 239).

This case illustrates that N_2 does not invariably cause complete pulp necrosis after a prolonged period of action.

Experiment 59. Figs. 240, 241, page 205.

N_2 had been placed in contact with the exposed pulp of the lower left second premolar for 6 months, and after this period the pulp was seen to be reduced to fibrous strands (fig. 240). Although the gross architecture of the periapical region appeared intact, there was light infiltration with round cells (fig. 241). Cementogenesis, however, appeared active and vital tissue was retained in some apical ramifications. There appeared to be stenosis of some of these apical channels with formation of new cementum.

Experiment 60. Figs. 242 - 245, page 206.

Following pulpectomy, the mesial root canal of the lower right 4th premolar was reamed and filed to the cemento-dentinal junction and immediately root filled with N_2, while the distal canal was similarly prepared and filled with root canal sealer. After 9 months, the radiograph did not suggest that bone loss had occurred over either apex (fig. 242).

Low power photomicrography showed that the distal apex was free from inflammatory cells (fig. 243) and under higher power, the apical ramifications were seen to contain vital tissue (fig. 244).

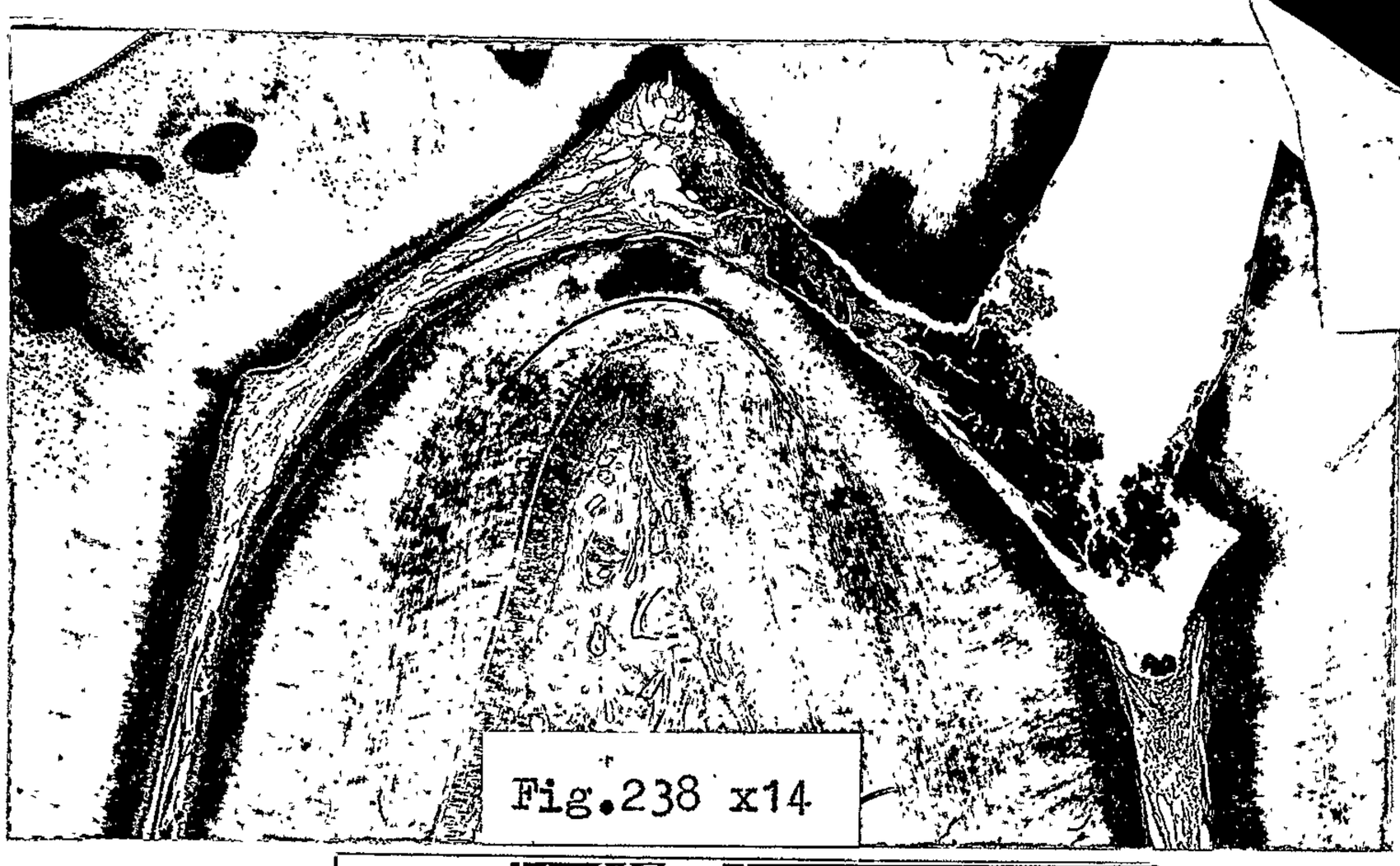

Fig.238 x14

Fig.239 x125

Fig.240 x9

Fig.241 x125

D. 3

Fig.242

Fig.243 x8

Fig.244 x32

Fig.245 x32

Mesially, around the apex of the canal filled with N_2, there were several foci of inflammatory cells (fig. 245) and the apical ramifications contained necrotic tissue. These aggregations of chronic inflammatory cells were not extensive and little bone resorption had occurred.

Experiment 61. Figs. 246 - 249, page 208.

The apices of both roots of the lower left 4th premolar were perforated and the mesial canal was filled with Gysi Trio paste together with a gutta percha point which was extruded through the apex. Distally root canal sealer was used to overfill the canal and again a gutta percha point was passed through the apex (fig. 246).

After 6 months the Gysi paste was still being cleared from the tissue. Scattered throughout the area and densely packed in some locations were macrophages which were engorged with paste (figs. 247, 248). The point lay free in the periapical tissue and was itself unattended by any inflammatory response. Some lymphocyte and plasma cell infiltration was seen near the root apex, but the inflammatory reaction must be regarded as very slight when the paraform content of the paste is considered.

Distally the gutta percha point was surrounded by a dense connective tissue sleeve (fig. 249). An inflammatory focus was seen near the apex.

Experiment 62. Figs. 250 - 253, page 209.

Following pulpectomy in the lower left 4th premolar, root canal sealer and a gutta percha point were inserted into the mesial canal while N_2 together with a gutta percha point were used to fill the distal canal. The radiograph obtained after 6 months suggested that both periapical areas were sound and there was no evidence of bone resorption (fig. 250).

Low power photomicrography showed that both periapical areas were free from inflammatory change (fig. 251) and higher power examination revealed the retention of vital tissue in the apical ramifications of both roots (figs. 252, 253).

Fig.246 x11

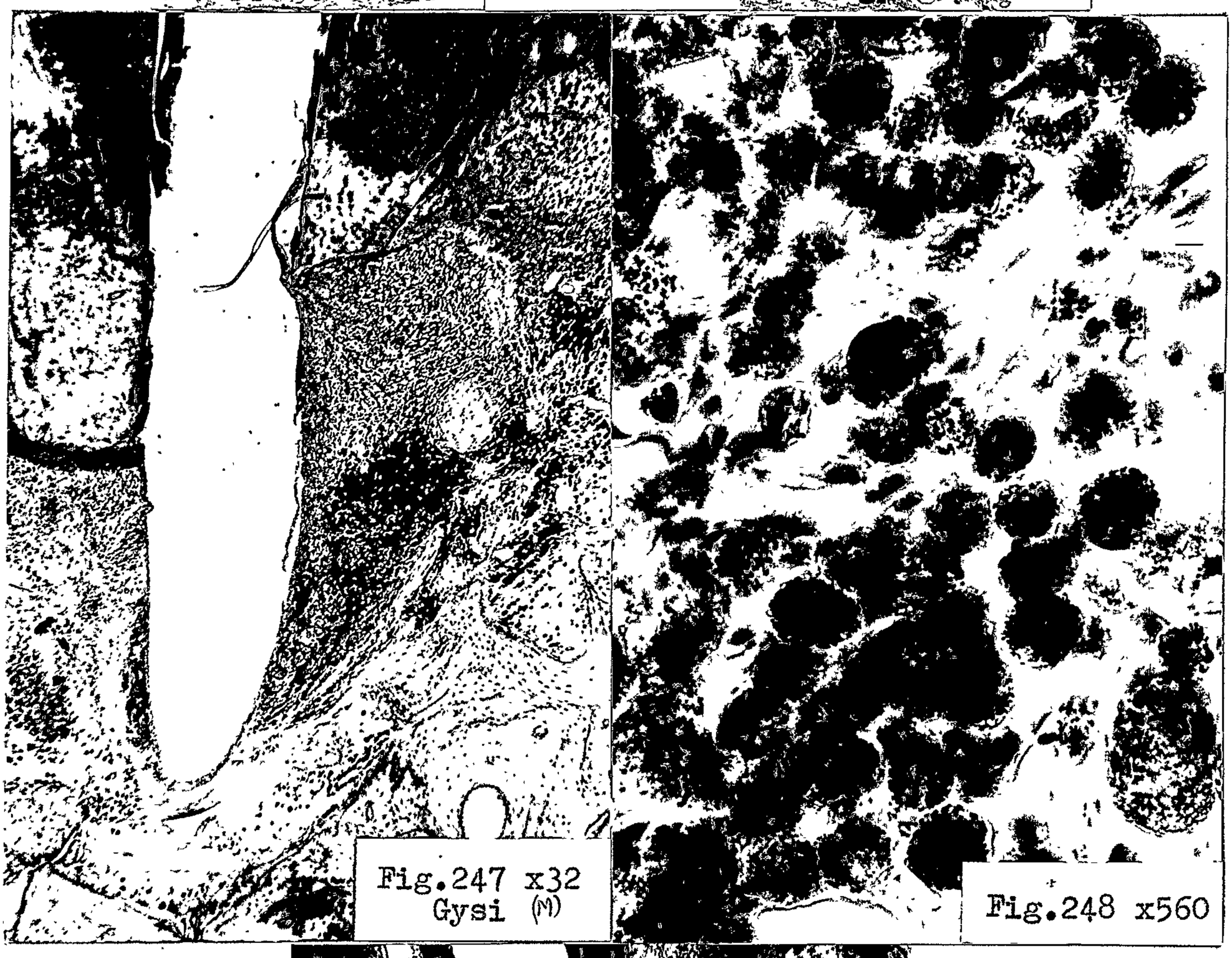

Fig.247 x32
Gysi (M)

Fig.248 x560

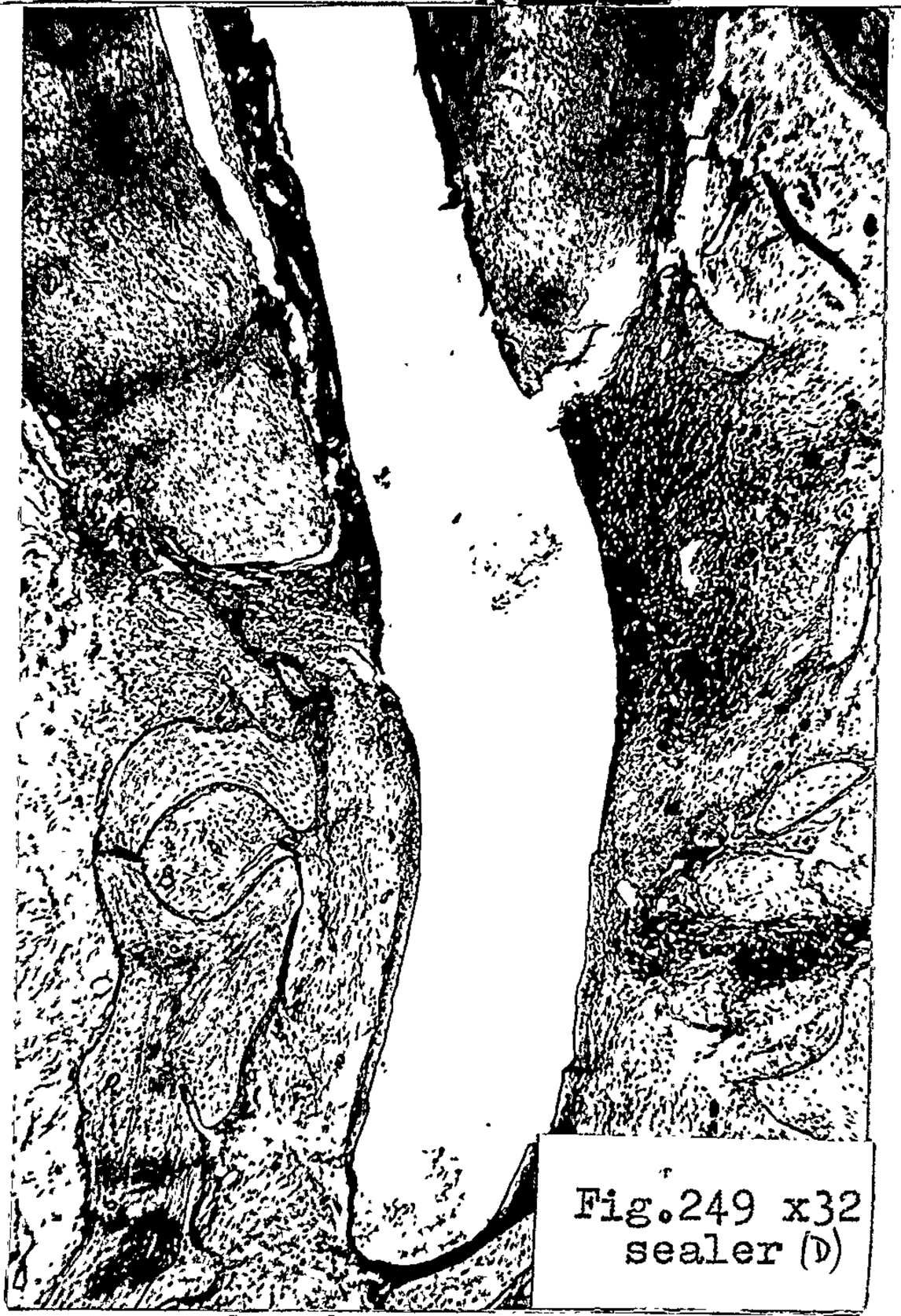

Fig.249 x32
sealer (D)

M D.

Fig.250

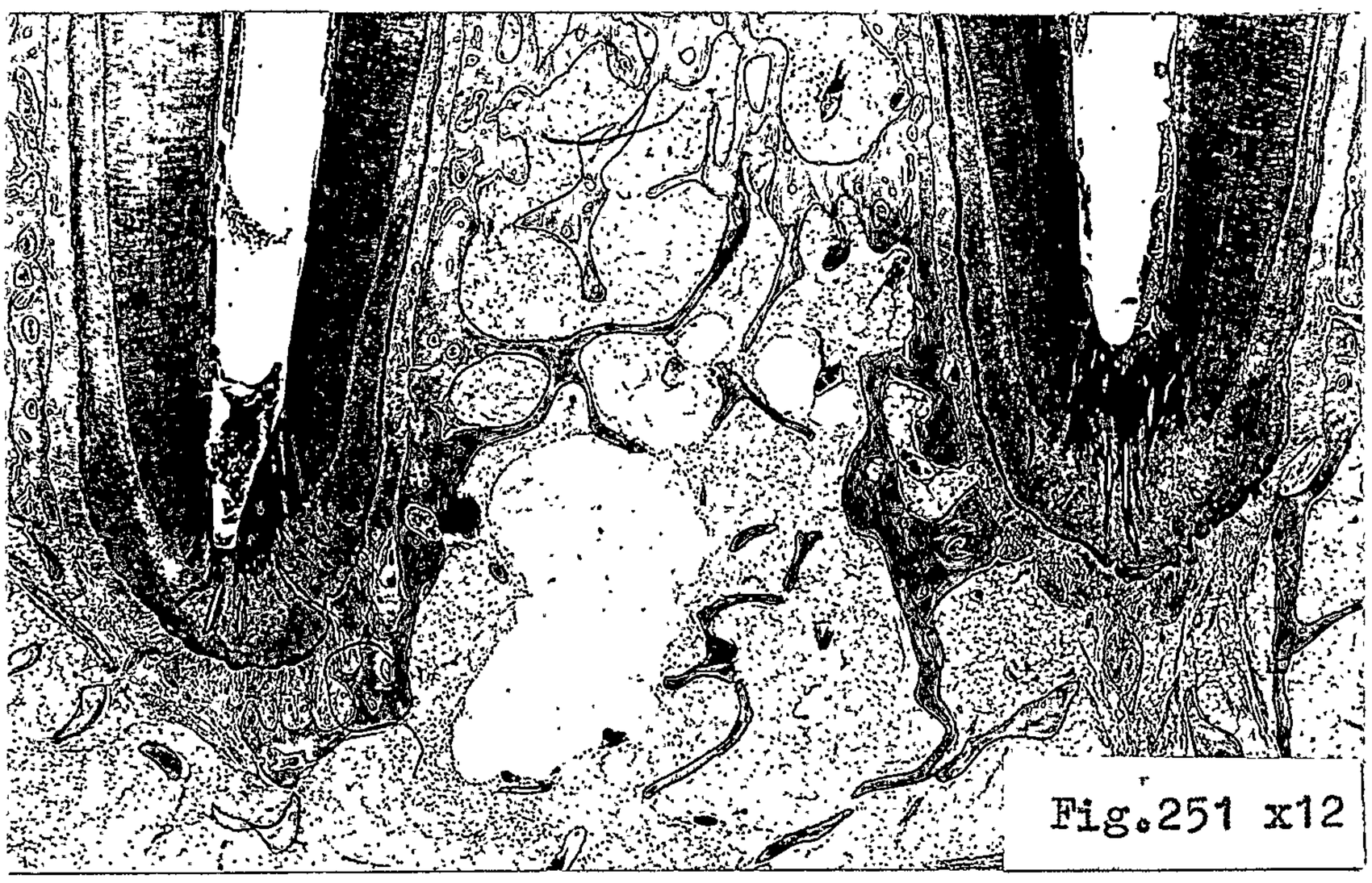

Fig.251 x12

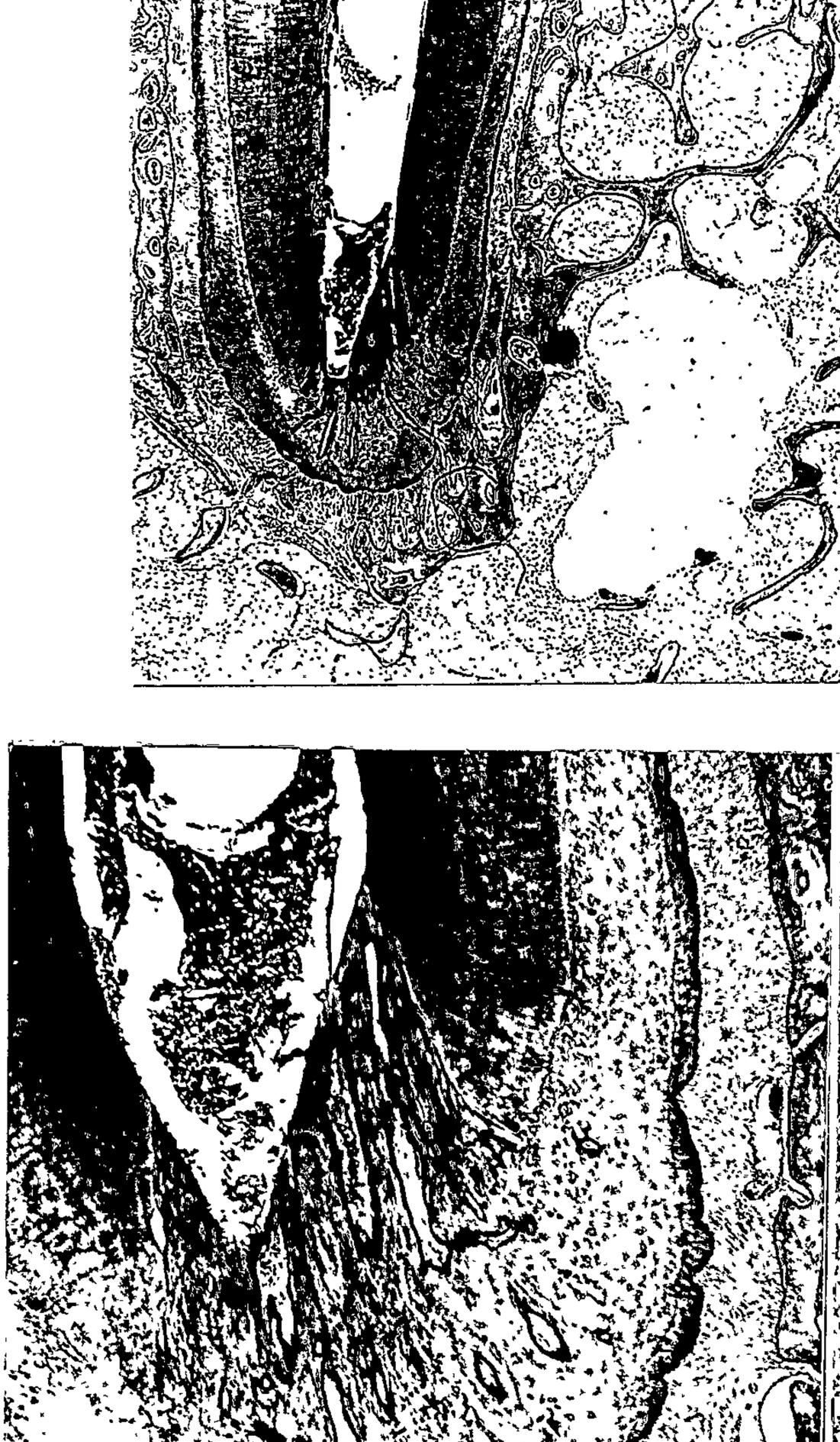

Fig.252 x25
mesial.sealer

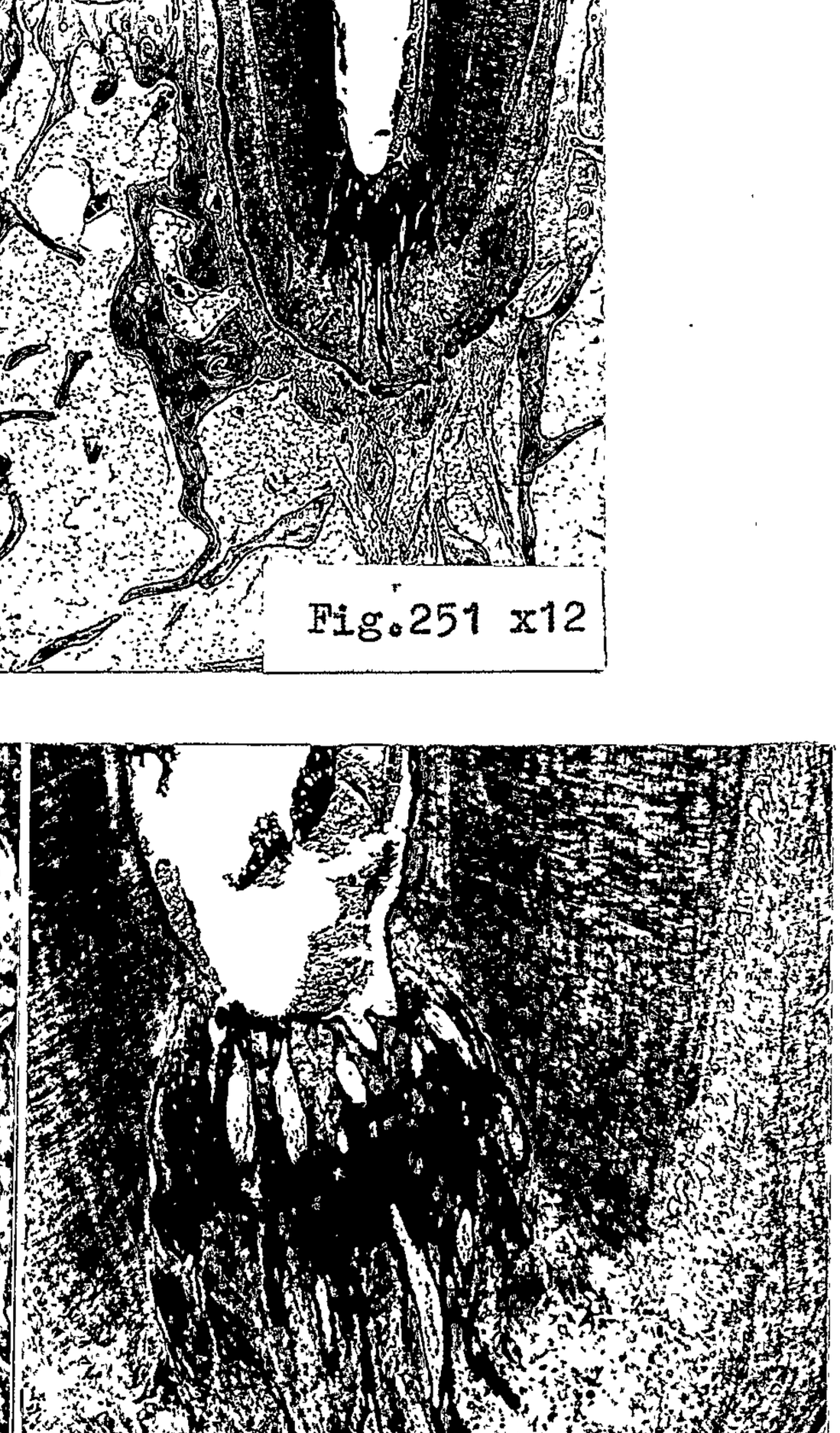

Fig.253 x25
distal.N2

Experiment 63. Figs. 254 - 257, page 211.

Both apices of the lower left 4th premolar were perforated and N_2 together with a gutta percha point was used to fill the mesial canal. Distally, root filling was effected with Gysi paste and a gutta percha point. The radiograph showed the root filling in each case to be approximately level with the apex (fig. 254).

After 14 months, an excellent picture of healing was observed around each apex. The N_2 paste was well tolerated by the tissue and vital bone remained in close proximity (fig. 255). A very small focus of round cells lay juxtaposed the paste near the apex, but the solidified N_2 material appeared to be relatively inert.

Distally, the Gysi Trio paste had been eliminated from the apical region of the canal, and a fibrous tissue sleeve had grown into the canal around the gutta percha point (fig. 256). A high power view of this encapsulating tissue is seen in Fig. 257. In the apical tissue, very small remnants of the paste remained, and these were being cleared by macrophages. Anchylosis of the root tip had occurred and this was not an uncommon event following perforation of a root apex. Vital tissue persisted in the ramifications.

Experiment 64. Figs. 258 - 261, page 212.

The apices of the roots of the lower left 3rd and 4th premolar were perforated. KRI paste was then extruded through each mesial apex while N_2 was passed into the periapical tissue through each distal apex. After 3 months, the radiograph showed that the KRI paste had been resorbed up each mesial root canal, but there was no macroscopic alteration in the amount or location of N_2 in the periapical tissue (fig. 258).

In one instance the N_2 cement was enclosed in dense connective tissue and the inflammatory response was not marked (fig. 259). In the other case, the extruded paste was not well encapsulated but the inflammatory reaction was again slight (fig. 260). Bony trabeculae in contact with the N_2 material lacked osteocytes and were probably necrotic, but healthy bone was apparent near the fibrous tissue capsule. Some resorption of N_2 was in progress as evidenced by the loaded macrophages (fig. 261) but this was evidently a very slow process. An unusual observation was the presence of vital tissue in

Fig.254

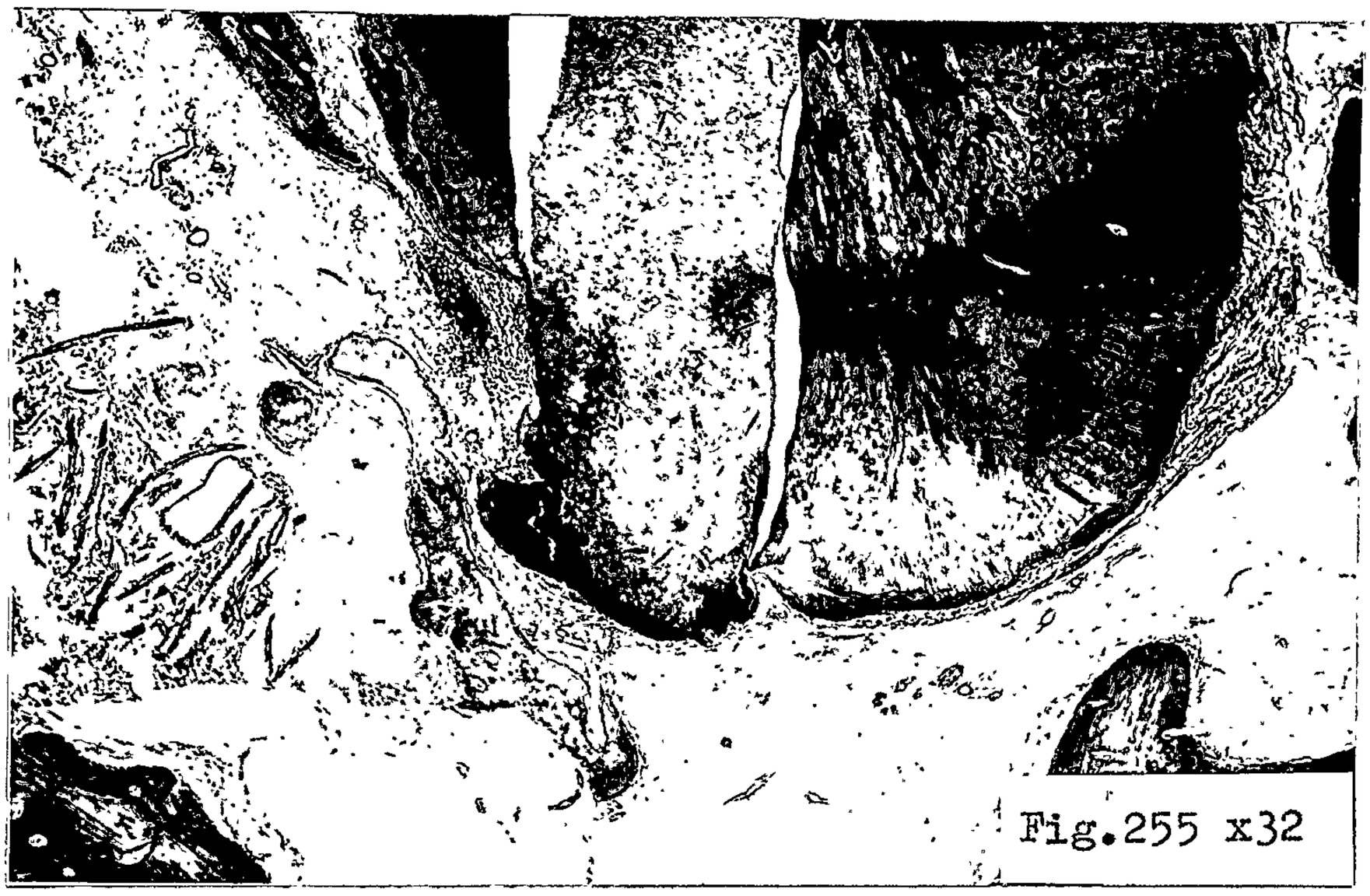

Fig.255 x32

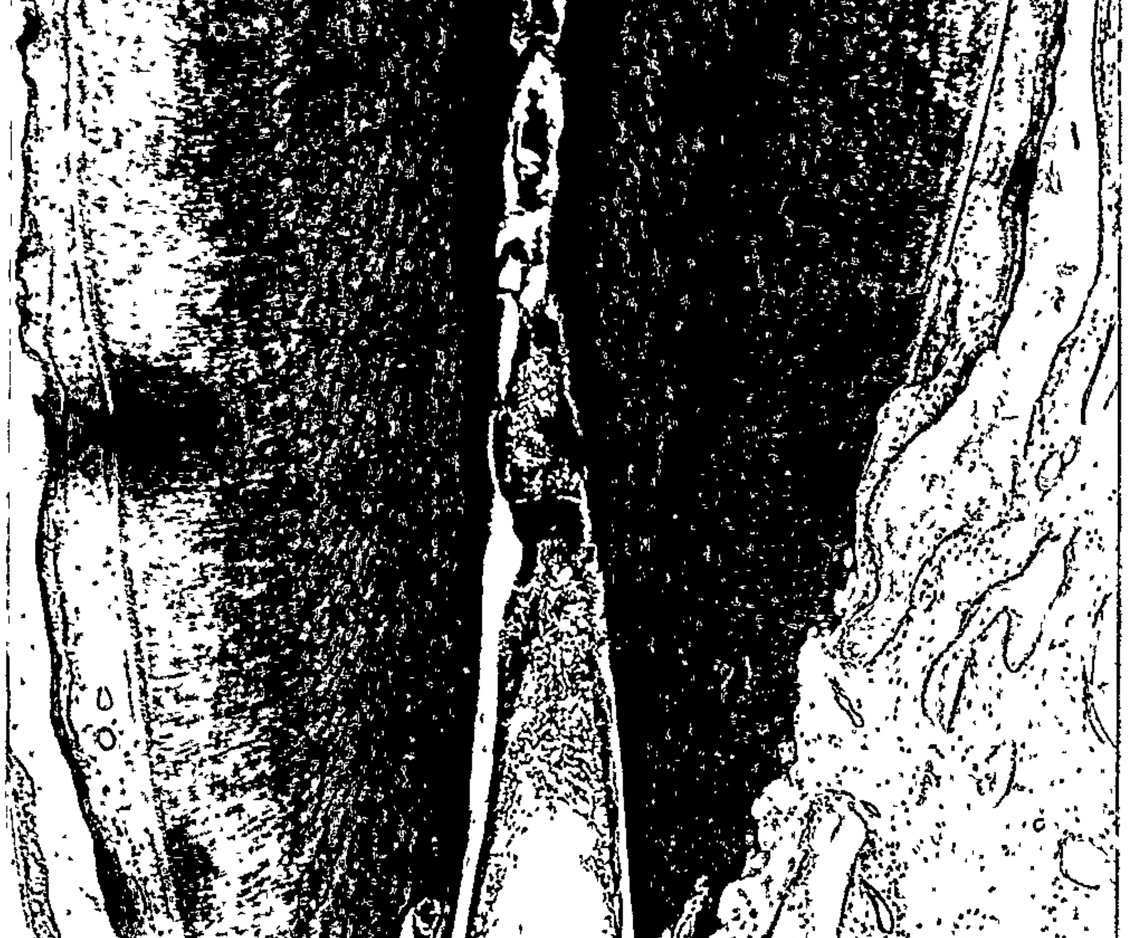

Fig.256 x25

Fig.257 x560

Fig.258

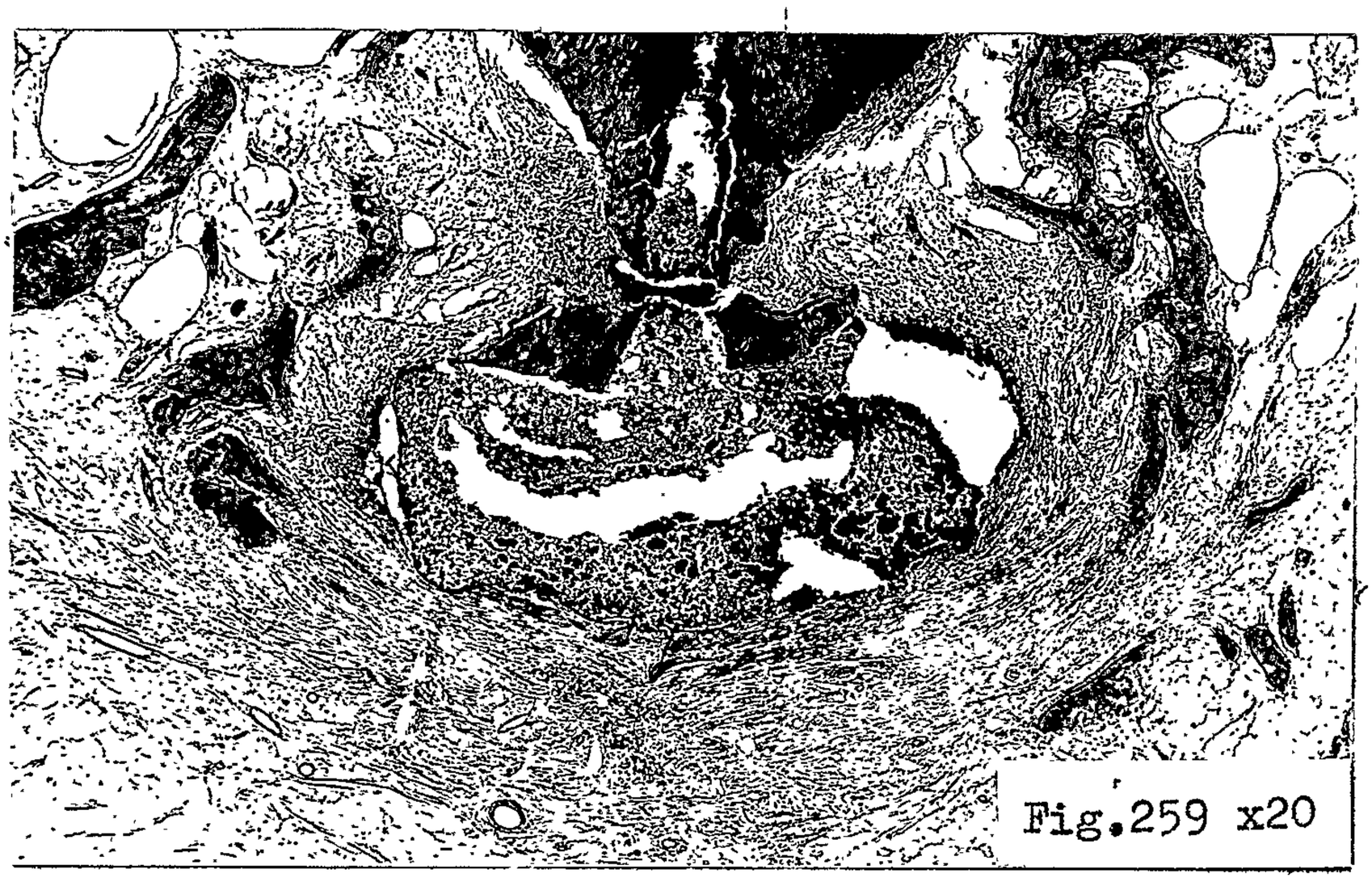

Fig.259 x20

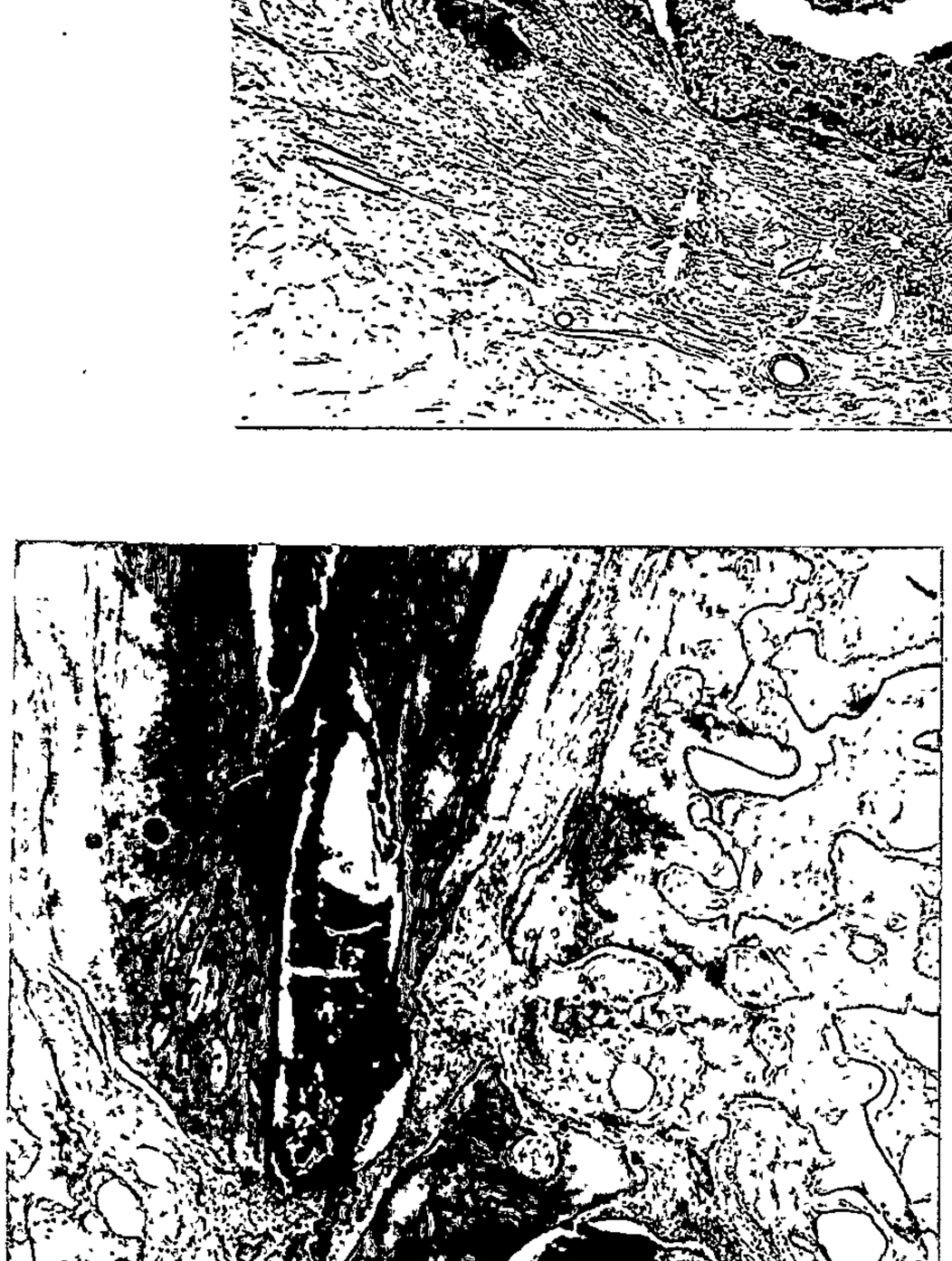

Fig.260 x18

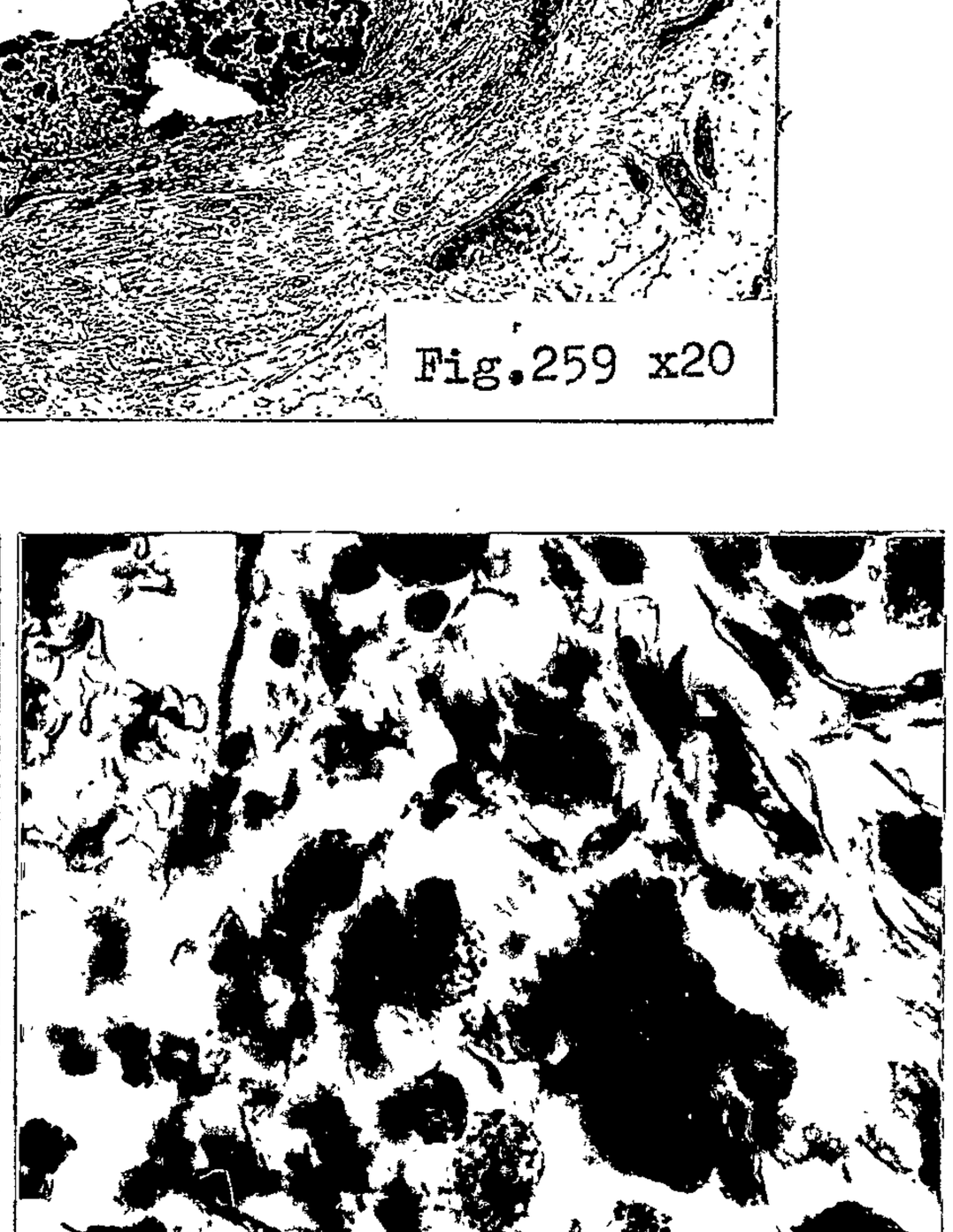

Fig.261 x560

some of the apical ramifications.

Experiment 65. Figs. 262 - 268, page 214, 215.

Both apices of the lower left 3rd premolar were perforated and eccially Gysi Trio paste was conveyed into the periapical tissue while distally Oxpara was similarly forced through the apex (fig. 262). After 3 months, the Gysi paste was seen to be resorbed into the root canal while some diminution of the Oxpara had occurred due to resorption (fig. 263).

In the periapical region below the mesial root, Gysi paste was still present, but it had all been engulfed by macrophages (figs. 264, 265). There was no inflammatory response. In some apical ramifications, vital tissue was retained.

Two photomicrographs of the distal periapical area revealed that the extruded Oxpara had evoked very little inflammatory change. (figs. 266, 267). The material was encased in connective tissue and large macrophages in the surrounding tissue contained quantities of particulate matter (fig. 268).

Experiment 66. Figs. 269 - 272, page 216.

Pulpectomy was performed in each root canal of the lower right 3rd premolar (e, fig. 269) but the apices were not perforated. N_2 was used to fill the mesial canal while Oxpara was inserted into the distal canal.

The periapical tissue over each root was normal and without any inflammatory change after 3 months. Distally, the Oxpara had not caused necrosis of tissue in many of the canal ramifications (fig. 270).

Some of the N_2 paste had infiltrated the apical ramifications and partly extruded on the root surface (fig. 271). This did not excite any inflammatory response. In all such cases the extruded paste was either encased in connective tissue or sealed off by layers of newly formed cementum (fig. 272).

Experiment 67. Figs. 273 - 276, page 218.

Following pulpectomy and preparation of the canals in the lower right 4th premolar (e, fig. 273), N_2 was used to fill the mesial canal while Gysi Trio paste was inserted distally. The root apices were not perforated.

Fig.262

Fig.263

Fig.264 x18

Fig.265 x125

Fig.266 x24

Fig.267 x22

Fig.268 x560

Fig.269

Fig.270 x24
oxpara

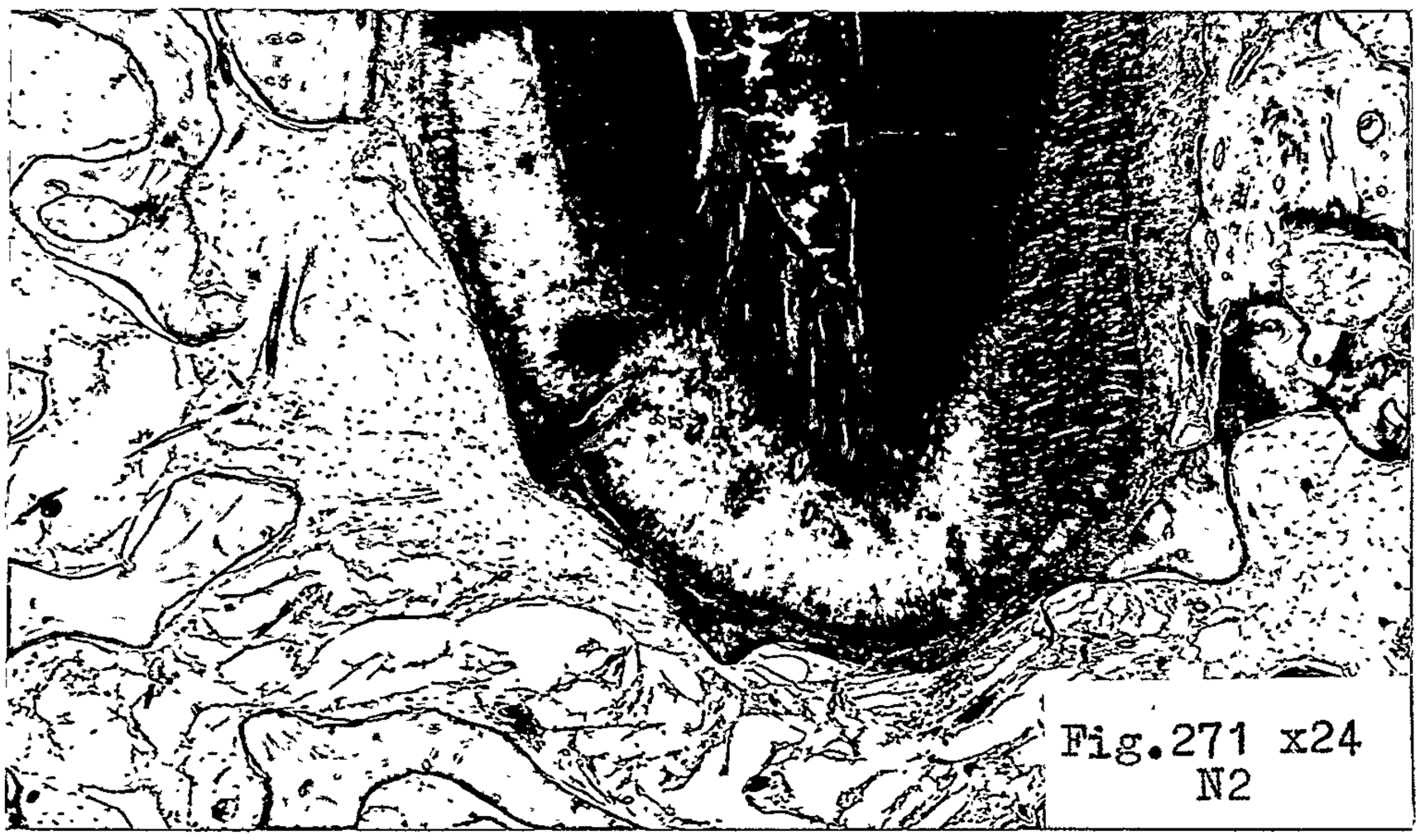

Fig.271 x24
N2

Fig.272 x125

After three months, the periapical tissues around each root appeared undisturbed (fig. 274 Gysi; fig. 275 N_2). Most of the distal apical ramifications possessed necrotic tissue as a result of the action of the Gysi paste, but periapical irritation had not supervened. As before, the N_2 paste had infiltrated several of the apical ramifications and was extruded into the periapex, where it had become surrounded by fibrous tissue or hard substance. Figure 276 shows the formation of bone-like tissue around some extruded paste and this had fused to the root surface.

Experiment 68. Figs. 277 - 279, page 219.

Periapical areas had been developed on both roots of the lower left 3rd premolar by streptococcus viridens inserted into the root canals for a period of 3 months. The mesial root canal was sealed off as control, while distally, immediate root filling with N_2 was carried out following canal debridement (fig. 277).

The radiograph taken at the time of sacrifice six months later showed marked reduction in the size of the distal granuloma, which would, on radiographic evidence alone be judged to have healed (fig. 278). On sectioning the tissue, however some residual inflammation was seen distally but this was considerably less in extent than the reaction around the mesial apex (fig. 279). Bacteria were stained in both apices. Extensive root resorption had occurred concomitant with the inflammatory process.

Fig.273

Fig.274 x20
Gysi

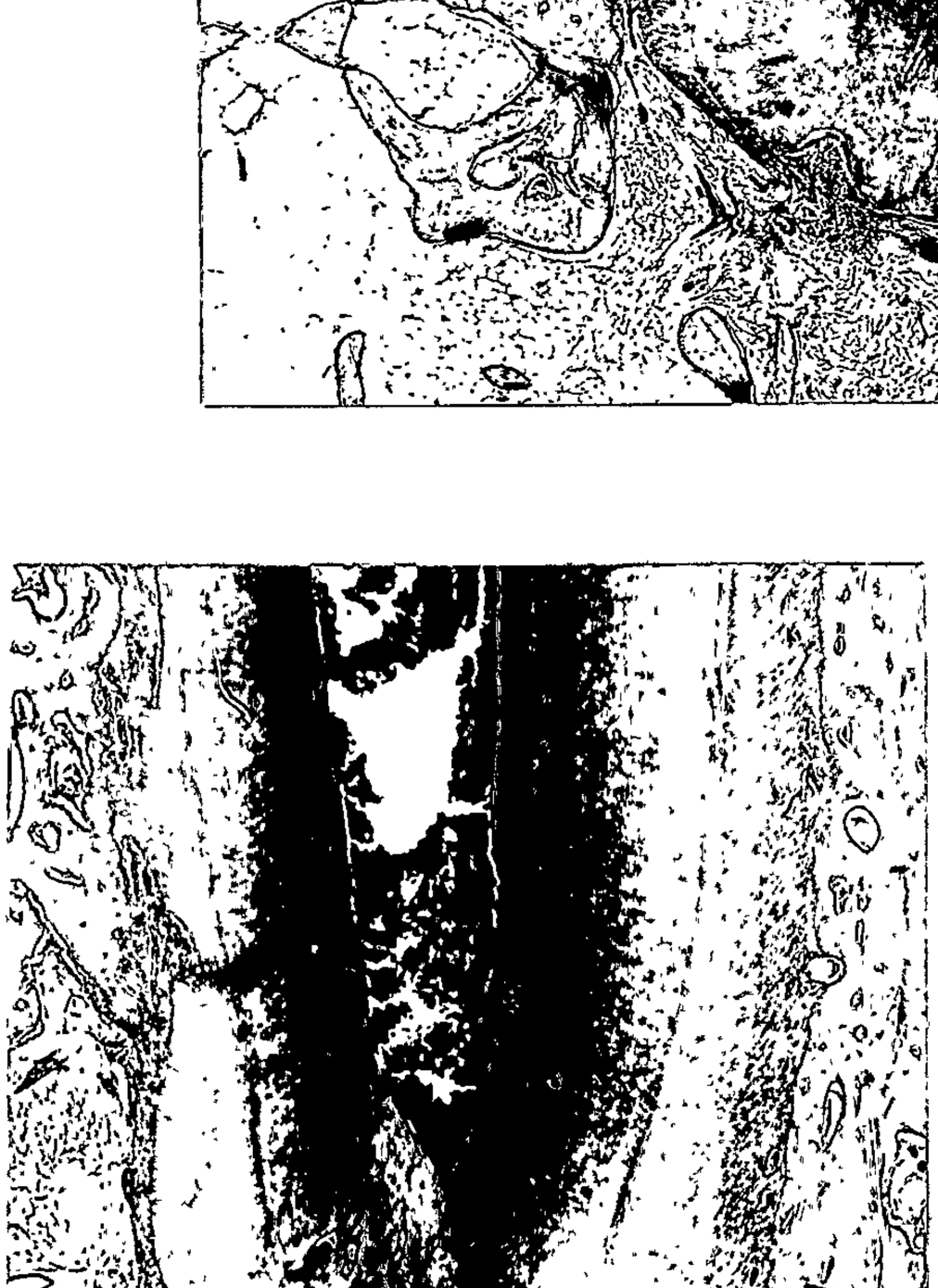

Fig.275 x19
N2

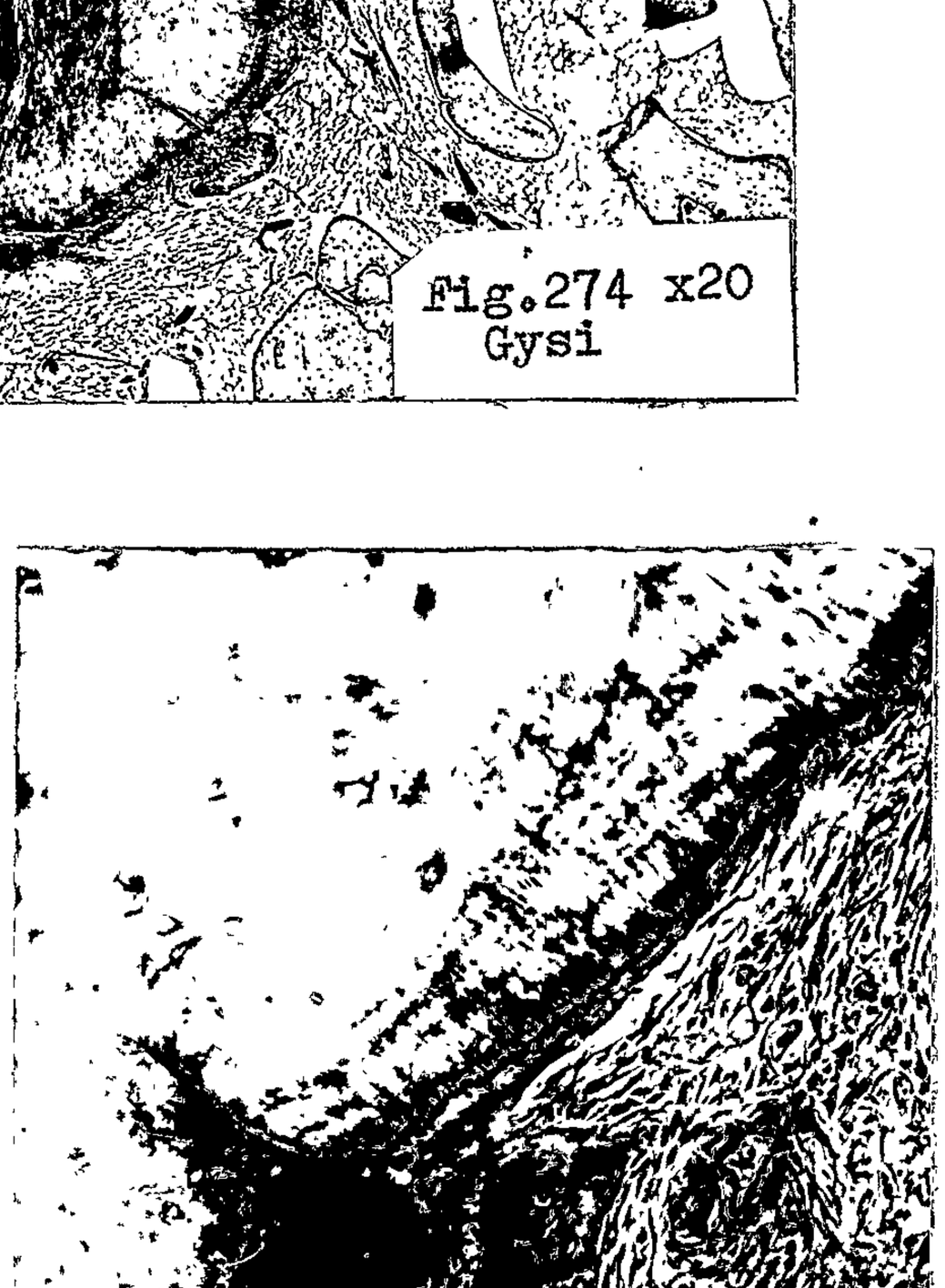

Fig.276 x125

Fig.277

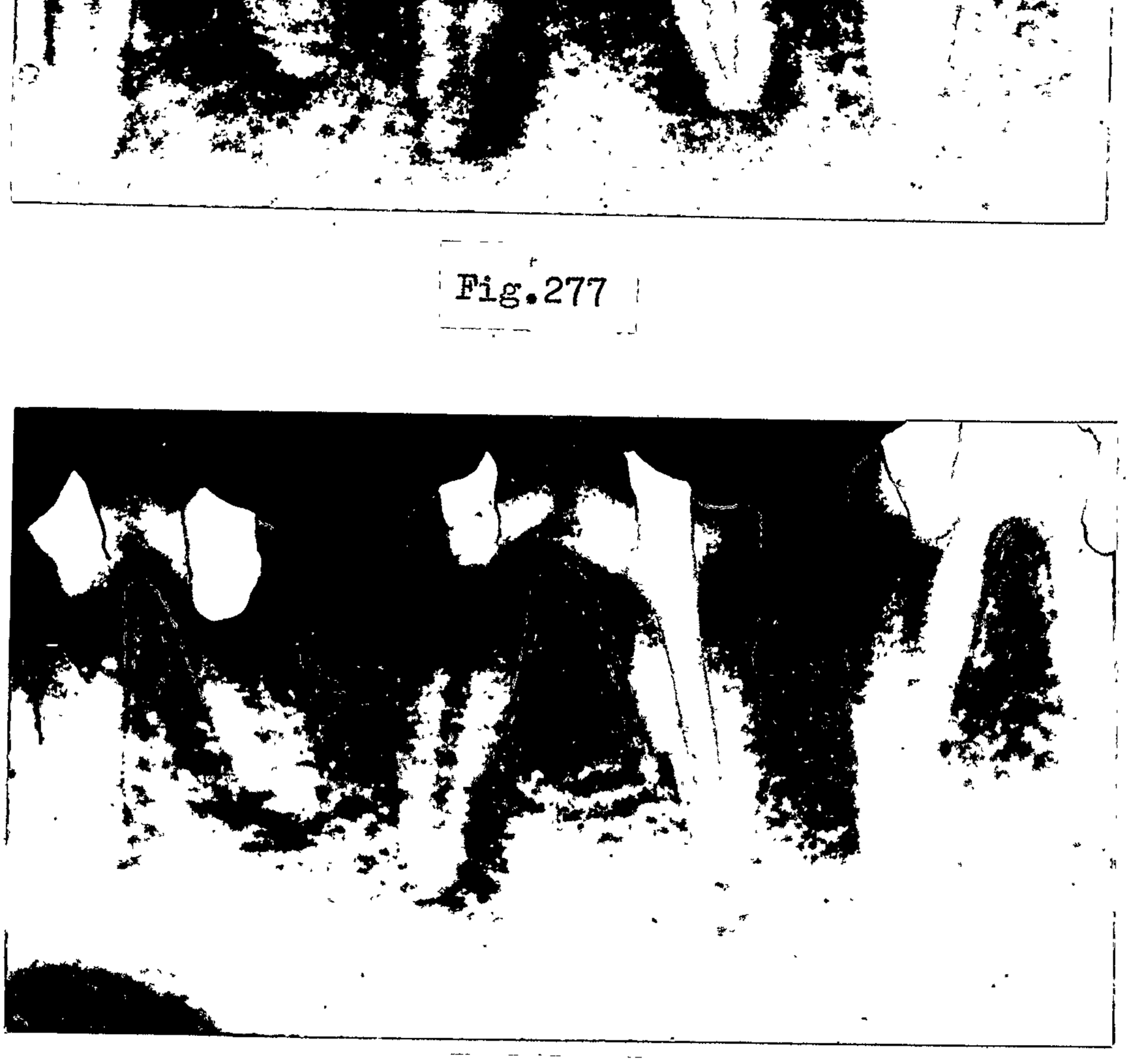

Fig.278

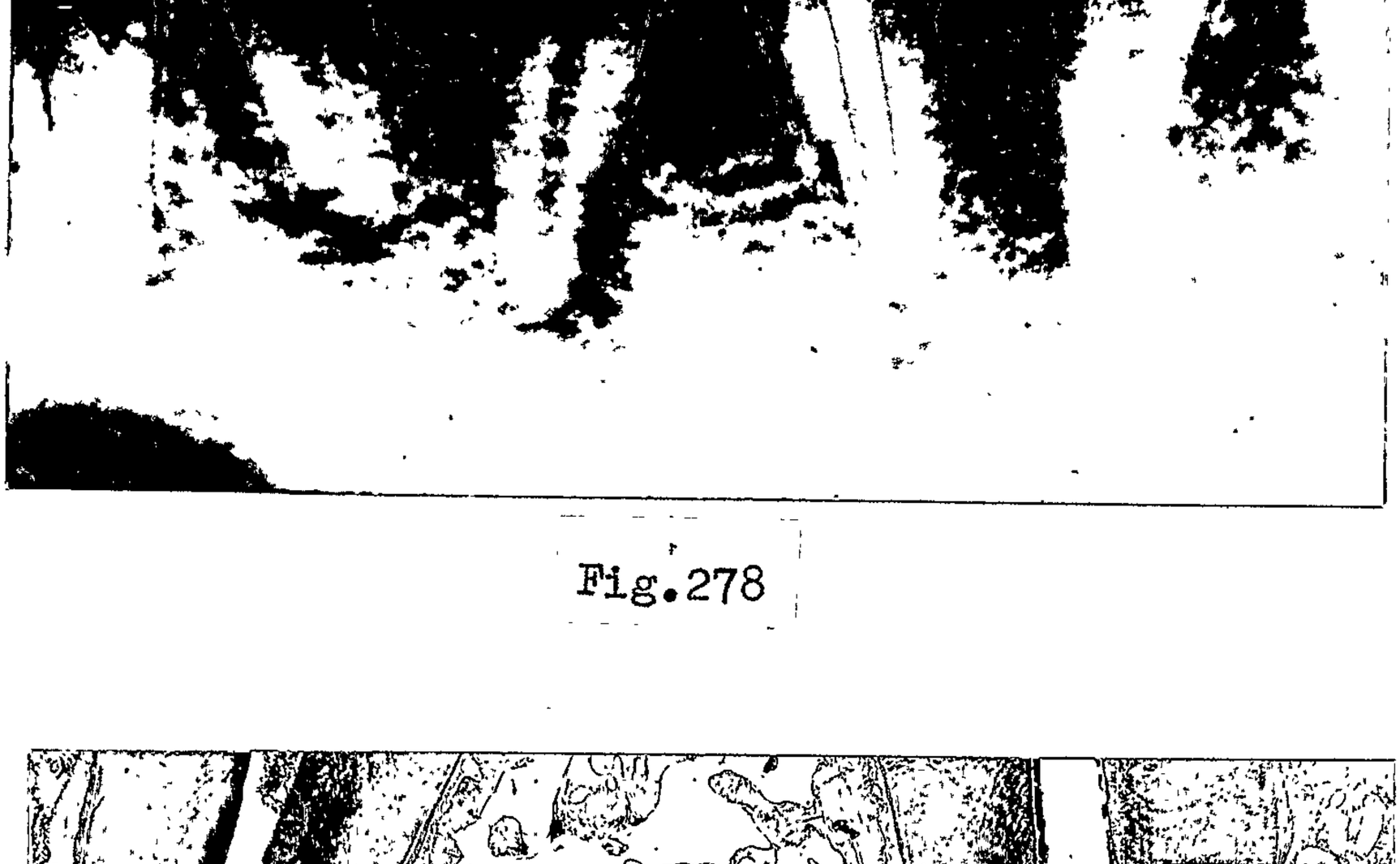

Fig.279 x10

Tooth treated	Treatment	Period of observation	Results
49. LL 3rd Pm	Pulpotomy in each root; N_2 inserted mesially; Oxpara distally.	7 months	Periapical inflammation distally but mesial apex undisturbed. Pulp reduced to fibrous strands in each canal.
50. LR 3rd Pm	Pulpotomy in each root; N_2 inserted mesially; Oxpara distally.	9 months	Periapical reaction slight around mesial root but moderately severe distally. Pulp in each canal reduced to fibrous remnant. Mesial canal walls lined with deposit of atubular dentine.
51. LL 2nd Pm	Distal pulp capped with N_2.	5 months	Periapical tissues normal. Pulp reduced to fibrous strands.
52. LR 3rd Pm	Mesial pulp capping with Oxpara; distal pulp capping with N_2.	5 months	Pulp degeneration in both canals. Slight periepical inflammation over both roots but bundles of dense fibrous tissue radiate from the distal apex.
53. LL 2nd Pm	Gysi Trio placed over pulpotomy wound.	14 months	Persistent apical irritation. Pulp completely lost.

54.	LL 3rd Pm	Mesial pulp cap with Gysi Trio; distally N_2 inserted into pulpotomy wound.	12 months	Pulp completely lost mesially and intense apical irritation. Fibrous pulp remnants distally with very slight apical disturbance.
55.	LR 2nd Pm	Pulpotomy in each canal; Gysi Trio inserted distally, N_2 mesially.	12 months	Pulp destroyed in each canal. Apical abscess distally and granuloma formation mesially, the latter containing proliferating epithelium.
56.	LR 3rd Pm	Pulp exposure in each canal. N_2 capping mesially, Gysi Trio distally.	2 weeks	Mesial pulp oedematous and degenerating. Distal pulp 'fixed' beneath wound but degenerating elsewhere.
57.	LL 3rd Pm	Pulp exposure mesially capped with ZnOE; distal cap with N_2.	2 weeks	Mesial pulp disorganised and haemorrhagic. Distal pulp degenerated and reduced to strands.
58.	LR 3rd Pm	Mesial exposure capped with N_2.	6 months	Vitality retained and pulp normal except for an area beneath the paste.
59.	LL 2nd Pm	Pulp exposure capped with N_2.	6 months	Pulp reduced to fibrous strands. Some vital tissue retained in ramifications. Very little disturbance of apical tissues.

✓ 60.	LR 4th Pm	Pulpectomy. Mesial canal filled to cemento-dentinal junction with N_2; distal sealer.	9 months	Distal apex normal and vital tissue in ramifications. Little bone resorption mesially with s ight periapical inflammation.
61.	LL 4th Pm	Apices perforated. Gysi Trio with GP extruded mesially; distally, sealer with GP extruded.	6 months	Gysi paste contained in macrophages but inflammatory cells few. Gutta percha point distally encased in connective tissue sleeve.
62.	LL 4th Pm	Pulpectomy. Mesial canal filled with sealer and GP; distal canal filled with N_2 and GP.	6 months	Both periapical areas undisturbed and vital tissue retained in ramifications in both apices.
63.	LL 4th Pm	Both apices perforated. N_2 with GP filled the mesial canal while Gysi Trio plus a GP point filled the distal.	14 months	N_2 paste we'l tolerated by periapical tissue. Gysi eliminated from apical canal and a fibrous tissue sleeve had grown into the canal around the gutta percha point.
64.	LL 3rd and 4th Pm	Apices perforated. N_2 extruded into periapex through each distal canal.	3 months	Inflammatory reaction slight. N_2 walled off by connective tissue capsule. Some loaded macrophages but no macroscopic evidence of resorption.

65. LL 3rd Pm	Apices perforated. Gysi extruded through mesial apex; Oxpara forced through distal apex.	3 months	Gysi paste resorbed into canal and some diminution in amount of extruded Oxpara. Loaded macrophages but very little inflammation in either situation.
66. LR 3rd Pm	Pulpectomy but apices unperforated. Root filling with N_2 mesially; Oxpara distally.	3 months	Periapical tissues normal. N_2 extruded through ramifications encased in connective tissue or covered with cementum. No inflammation.
67. LR 4th Pm	Pulpectomy but apices unperforated. N_2 filling mesially; Gysi Trio distally.	3 months	Periapical tissues undisturbed. N_2 extruded through ramifications surrounded by hard tissue.
68. LL 3rd Pm	Apical areas produced by streptococcus viridens. Mesial control; distal root filled with N_2 to cemento-dentinal junction following debridement.	6 months	Radiographic evidence of marked reduction in size of distal granuloma but some residual inflammation. Reaction around mesial apex severe.

Discussion

Experiments with the paraformaldehyde pastes Oxpara and Gysi Trio paste indicate that they cause necrosis when applied to vital dog pulp and in every case studied in this series, periapical inflammation supervened following pulp death. This apical disturbance was frequently severe. N_2 also causes necrosis and usually results in the reduction of pulp tissue to fibrous strands, although in one of the ten pulp capping experiments pulp vitality was retained. In one other case N_2 applied to pulp tissue had apparently caused the elaboration of atubular dentine along the canal walls with stenosis of the root canal. Pulp degeneration had supervened however. Although the action of N_2 on the pulp is usually necrotising, the material did not cause marked periapical inflammation following pulp death and in some instances vital tissue persisted in the canal ramifications. Light infiltration of the periapical tissues with round cells was sometimes observed but in no instance was the apical disturbance as severe as with Gysi or Oxpara. In some cases, pulp death induced by N_2 was followed by the generation of a marked fibroblastic response in the periapical tissues. In one instance, epithelial proliferation in an apical granuloma was observed.

The use of these pastes as a root filling material in this pilot survey did not demonstrate any advantages over zinc oxide based root canal sealer. Their effectiveness in sterilising canals, however, did not receive close attention. Neither Gysi Trio nor Oxpara are permanent when extruded into the periapical tissues, and the former material is rapidly cleared from the area by macrophages. An interesting observation was the absence of any marked inflammatory response attending the extrusion of these materials into the apical tissues. Provided there is free access to an irritant substance the defence mechanisms in periapical tissues appear well equipped to counteract or minimise the disturbance.

N_2 occasionally causes slight apical irritation when used to fill root canals, but this is not invariably the case. In some experiments the material was well tolerated when extruded into the periapical tissue and encapsulation in fibrous tissue occurred. When

in contact with bone, necrosis supervened, but trabeculae in close proximity were unaffected. The material is resorbed to a certain extent by macrophages, but the process is very slow and the cement must be regarded as relatively permanent when passed into the periapical tissues. Small quantities of cement which flowed onto the root surface through apical ramifications eventually became sealed over by hard tissue. It is probable that this event occurred after the cement had solidified in which state it appears relatively inert.

In the single experiment in which an infected root canal was filled with N_2, regression of the apical reaction occurred, but again, this outcome did not seem significantly different from that which occurs when zinc oxide based sealer is used.

These dog experiments indicate that N_2 is less irritant than other paraformaldehyde pastes in common use, but it nevertheless causes destruction of pulp tissue. In the dog, pulp destruction was usually complete, but examination of treated human pulp tissue is necessary to determine whether the pulp in man is similarly vulnerable to the action of this material.

SECTION 7

Some Clinical Implications

Caution must be exercised when attempting to extrapolate findings gained from animal experimentation to the human situation. The pulp tissue in the dog appears to be more prone to degeneration, following injury, than human pulp, while the defence mechanisms in the periapical tissues are probably as vigorous as in the human. Also, the anatomy of the dog apex is considerably more complex than in the human and there are differences in the process of healing following root canal therapy.

Bearing these points in mind, certain findings in the dog may be reviewed and the possible clinical implications discussed. In few instances can definitive conclusions be reached and further investigations must be pursued to elaborate on the observations made in this present study, a study which was primarily concerned with evaluating the dog as a suitable animal for endodontic research.

Immediate r.ot filling following vital pulpectomy

It is common practice in clinical endodontics to carry out pulpectomy and canal enlargement and then wait for a short period before undertaking root filling.

Following severance of the pulp, it has been shown in humans and monkeys that an inflammatory response, initially acute, persists in the apical tissues for periods up to one month. It is argued that immediate root filling would prevent drainage from the oedematous apical tissues, cause extrusion of the tooth and precipitate a painful and prolonged apical periodontitis.

The proponents of immediate root canal filling, however, maintain that immediate obturation obviates the possibility of contamination during reopening of the canal, and that the sealing of the canal covers the wound and hastens healing. Extrusion of the tooth is counteracted by relieving the tooth of occlusal stress.

In the dog experiments where canals were immediately root filled to the cemento-dentinal junction using zinc oxide based root sealer and gutta percha, complete healing had occurred after six months and vital tissue was retained in the apical ramifications.

The long term results of immediate root filling may therefore be regarded as satisfactory.

Short term experiments in dogs are required however, to observe the extent and duration of the apical disturbance in filled and unfilled canals. An assessment of whether pain symptoms are any more prolonged or intense after immediate root filling than after instrumentation alone can only be made in the human, and such clinical observations together with further dog investigations may lead to a reassessment of the necessity for delayed root filling.

Root canal therapy complicated by overfilling with zinc oxide based cements and gutta percha

Following accidental extrusion of gutta percha points or root filling paste into the periapical tissues, consideration is frequently given to the need for apical curettage. In the absence of symptoms, however, and in situations where this operation is not readily undertaken, it is probable that these materials may be left without danger of future complications in the majority of cases.

Dog experiments strongly indicate that a gutta percha point alone is well tolerated by the apical tissues and usually becomes encased in a sleeve of connective tissue. The amount of surrounding fibrosis is probably dependent on tooth mobility, and it is conceivable that hypermobility may lead to peripheral bone resorption around the point. If such a radiolucency is seen circumscribing an extruded point, however, it is unlikely to be due to an active inflammatory reaction.

While some reports suggest that zinc oxide and eugenol cements with or without silver are toxic to the periapical tissues, and some clinicians claim that overfilling leads to a higher failure rate in human root canal therapies, the reaction of the periapical tissues in the dog experiments to zinc oxide based root sealer was minimal. Vital bony trabeculae persisted in close proximity to the cement and the initial inflammatory response was transient. Fibrous encapsulation finally occurred. Very little phagocytosis was observed however, and the material deposited in the tissues must be regarded as relatively permanent.

The use of parenteral antibiotics in the treatment of infected canals

The eradication of bacteria from cemental lacunae in the dog apex by intracanal medication may prove to be impossible. Some dog experiments, however, tend to support the findings of Maysumiya and Kitamura, namely, that in favourable circumstances, bacteria in protected sites eventually die out following obturation of the main canal, and this reduction is probably due to diffusion of antibacterial substances from the inflammatory exudate. In one experiment reported on page 50 parenteral penicillin was administered following the filling of infected canals and bacteria could not subsequently be located in either the control or treated apices. It is tempting to speculate that the administration of this antibiotic enhanced the antibacterial action of the exudate and helped sterilise the infected apices. Further dog experiments using various bacteria which are either sensitive or resistant to a particular antibiotic may clarify this contentious issue.

Infected cementum apparently does not occur in humans, but bacteria invade protected sites such as necrotic debris in canal ramifications and tend to reinfect the main canal from these locations. It is not known whether parenterally administered antibiotics are able to reach these sheltered bacteria. Clinical studies are required to supplement dog experimentation, and these could take the form of root canal obturation under a cover of a selected antibiotic in cases where routine intracanal medication has proved ineffective. A careful analysis of the flora in the infected canal would be necessary together with sensitivity testing using a range of antibiotics.

The use of resorbable pastes in endodontics

When root filling a canal with an immature apex, the use of resorbable paste appears advisable in view of the probability of overfilling. Large quantities of this iodoform material have been seen to undergo very rapid removal from periapical tissues in the dog and ingrowth of healthy connective tissue into the apical part of the canal has been demonstrated. The extent of this tissue ingrowth appears to be limited, however and does not always reach the level of the unresorbed paste. Unsupported paste has also been seen to

fragment and it appears that reinforcement of the paste in the canal by a gutta percha point or the sealing of the incisal two thirds of the canal with a hardening cement is desirable.

Several observers have formed the opinion that resorbable paste, extruded into a periapical area, leads to the rapid replacement of granulomatous tissue with normal tissue and promotes the formation of bone. Indeed, two dog experiments (14 and 16) illustrate that healing of an apical focus can occur following treatment with resorbable paste even though the streptococcus viridans continues to reside in cemental lacunae. The above hypothesis must be explored in further dog experiments. Unhealed apical areas which have persisted following conventional root filling with zinc oxide based cement could be reopened and filled with KRI paste. Subsequent healing could then be compared with controls. Again, apical areas could be treated initially with KRI or root canal sealer and the difference in rate and extent of repair followed at regular intervals.

The dual mechanism of paste removal from the tissues which has been postulated in the present study requires confirmation and again the dog appears a suitable animal for such investigations.

<u>The use of calcium hydroxide</u>

The reaction of pulp tissue to calcium hydroxide has been extensively studied, but this does not imply that the variation in reaction has been fully documented. Its mode of action in stimulating dentine bridge formation has not been definitely explained, and the effectiveness of the material in promoting healing is markedly diminished when the pulp is seriously involved. The use of calcium hydroxide on exposed uninfected dog pulp resulted in dentine bridge formation in two cases, but pulp disintegration had occurred in each instance beneath the barrier. Pulp death has also been reported in human teeth following calcium hydroxide application and dentine bridge formation, and it may be misleading to promptly label the pulp disorganisation in the dog as artifact due to inadequate fixation. Further investigations may indicate that the elaboration of a dentine bridge in dog or human does not necessarily denote the preservation of pulp vitality.

A unique finding in three dog experiments was the rapid appearance of a very dense radiopaque line or plug beneath the calcium hydroxide. This was not a hard tissue barrier but a basophilic zone of cellular degeneration which had apparently become impregnated with calcium salts. The possibility of this response occurring in the human cannot be dismissed and careful reappraisal of postoperative radiographs depicting 'bridging' is required to distinguish between the moderate radiopacity of hard tissue and the densely radiopaque plug of necrotic cells. The status of the pulp beneath this layer requires further long term studies.

Some clinicians advocate the use of calcium hydroxide pastes as a root canal dressing following total pulpectomy. It is further stated that this material exhibits an osteogenic potential and is occasionally used as a temporary root filling in teeth with large periapical areas in order to bring about resolution. Experiments in the cat and a pilot experiment in the dog suggest that the material is irritant to the periapical tissues when confined in the root canal. Apical perforation and the insertion of calcium hydroxide directly into contact with the periapical tissues would provide valuable information as to its tissue compatibility and osteogenic potential. The process of healing following injection of calcium hydroxide and KRI into apical areas could also be compared.

<u>The use of glucocorticosteroid/antibiotic materials</u>

Application of one of these materials, Ledermix, to vital exposed human pulp appears to cause little disturbance and evokes a moderate calcific response. Dog pulp shows a slightly wider range of response but pulp vitality was retained in those cases where the cement was flowed over a single exposure in a tooth. This demonstration of tissue compatibility is encouraging, but to expect the material routinely to eradicate all bacteria from an infected pulp and concomitantly to allay inflammation and promote healing is optimistic and probably unrealistic. In view of the fact that it is clinically impossible to diagnose precisely the degree of pathological involvement of a cariously exposed pulp, and also impossible to determine the identity of the infecting organisms, the application of

Ledermix to all carious exposures must be regarded as empiric therapy.

It has been demonstrated however, that healing of some small carious exposures following the use of this drug can occur, and it is very probable that substantial clinical trial will be given this form of treatment in cases when root canal therapy is impractical. This is supportable only if the treatment is regarded essentially as a temporary measure and clinical tests are undertaken at regular intervals in an effort to establish that vitality has been retained. In this regard it is of interest to recall a case of hyperaemia treated with Ledermix, which after 653 days continued to give positive vitality responses but on histological section was found to be degenerate and chronically inflamed in the incisal half of the pulp[11].

It is possible that preliminary treatment of small vital exposures with Ledermix paste followed by pulpotomy and application of Ledermix cement to the cut pulp will result in a higher success rate than simple capping and a clinical and histological study along these lines appears to be urgently required.

It must be allowed that this combination of a corticosteroid with an antibiotic is a rational approach to the vexing problem of the carious exposure and it is interesting to speculate that future research may lead to the development of a more effective compound of this type. Selection of each ingredient must be undertaken with due regard to compatibility with the pulp tissue. For instance, the intense necrotising and irritating action of the hydrocortisone preparation Cortril when in contact with dog pulp and periapical tissue is an example of the danger inherent in labelling all corticosteroids in their various vehicles as anti-inflammatory.

Pilot dog experiments in which the Ledermix paste was used in infected canals did not produce conclusive results. Even though the infecting bacteria were sensitive to the paste in vitro, they were not always eradicated from canal ramifications, tubules and lacunae. Sometimes the combination of canal preparation, insertion of Ledermix paste and root canal filling did lead to healing of apical areas, but apical inflammation was not always counteracted by the paste. In one experiment the apical inflammation was allayed

but osteogenesis was inhibited and repair mechanisms suppressed.

In the present state of our knowledge, it appears that this paste may be of value in treating the symptoms of apical periodontitis, but the compound cannot be relied upon to eradicate bacteria from infected canals or routinely stimulate apical repair.

The use of formaldehyde preparations

Reports in the literature suggest that a proportion of pulps or root canals treated with formaldehyde preparations are clinically silent and successful. The formocresol pulpotomy technique in particular has attracted many adherents and histologic examination of treated primary teeth reveals that pulp vitality is maintained in the apical region in many instances. In such cases the limited period of action of the formaldehyde solution did not cause total pulp necrosis and it must have exerted an efficient sterilising action on the superficial pulp tissue. The advocates for N_2 imply that a similar situation exists when this material is used partially to fill root canals. Pulp remnants are exposed to the action of paraformaldehyde in low concentration for a short period until the paste solidifies, and only superficial necrosis supervenes, the deeper tissues remaining vital.

However, much depends on the resilience of the pulp tissue in any particular case. In the dog, total pulp necrosis occurred in nine out of ten experiments when N_2 was applied to exposures. While it is the considered opinion of the writer that dog pulp succumbs to trauma or irritation far more readily than the average human pulp, it is quite probable that the range of human variations include pulps of similar vulnerability. Short root fillings or treatment of pulpotomy wounds using N_2 very probably result in total necrosis of the residual pulp in a proportion of cases.

The dog experiments illustrate that an intact lamina dura on the radiograph of a treated tooth does not signify that a healthy pulp has been retained. The necrotising and irritant effect of N_2 applied to the pulp appears to be limited to the pulp canal, and periapical disturbance is so mild that bone resorption is not seen on a radiograph. In a clinical situation, the normal radiograph

would suggest successful healing and be so labelled. It is true that necrotic residues may remain clinically silent for an indefinite period, but the possibility of future complications cannot be overlooked when using formaldehyde preparations such as N_2.

Complete canal obturation with N_2 does not seem to offer any advantage over the zinc oxide based cements and its anti-bacterial properties have not been assessed. When extruded into the periapical tissues it is relatively permanent and dog experiments indicate that encapsulation in fibrous tissue may eventually occur. Clinical reports of pain following overfilling cannot be ignored, however, and if this material must be used, every effort should be made to confine it to the canal.

Other formaldehyde preparations such as Gysi Trio pasts and Oxpara have been in use for many years and have their adherents. Dog experimentation suggested that they are far more necrotising and irritant than N_2 when applied to pulp tissue and severe apical irritation with bone resorption indicated continued activity of the pastes over a long period. When extruded into the periapical tissues however, these materials were quickly phagocytosed, and when ingested by cells, there was no evidence of irritation or inflammation. Unless further work indicates that these materials possess significant sterilising properties when used in infected canals, their use in modern dentistry will be limited.

Conclusions

1. The position, pulp anatomy and size of dog mandibular premolar teeth permits a wide range of endodontic experimentation. Histologic section of teeth in situ allows observation of pulp and periapical response to endodontic procedures.

2. Dog pulp appears to be more sensitive to injury than human pulp and experiences greater difficulty in healing following treatment. This is regarded as a favourable feature in an experimental animal, for successful treatment of dog pulp may imply that the therapeutic measure is acceptable in the human situation.

3. In pulp capping experiments, application of a medicament to a single exposure in any one tooth is recommended. The disruption caused by pulpotomy in each canal may lead to total pulp necrosis no matter what treatment is instituted. Exposures in contralateral teeth provide the experiment with a control. A wide range of pulp reactions have been recorded following capping with various materials.

4. Pulpectomy may be carried out in both canals in the one tooth and periapical response compared over each apex following differing root treatments. The pulp in the teeth of the dog is readily extirpated, and an attempt to achieve partial pulpectomy is prone to result in total dislodgement.

5. The ability of dog periapical tissue to counteract or minimise irritation caused by instrumentation or extrusion of materials through the apex is probably as efficient as in the human. The encapsulation, phagocytosis or clearance of extruded material via the lymph channels is readily studied in the dog.

6. The complexity of the dog root apex is considered a desirable feature, for the retention of vital tissue in the multiple apical ramifications following root canal therapy procedures suggests that successful healing would similarly occur in the less complex human apex. The resorption and remodelling observed in human apices following root filling does not occur in the dog unless the animal apices have been mechanically perforated.

7. Following experimental infection of dog root canals, bacteria spread not only into tubules and canal ramifications but also into

cemental lacunae. This has not been observed in the human. Elimination of bacteria from these sites by tissue defences or by intracanal or paranteral medication is a problem in the dog. Measures which do eradicate bacteria from these protected locations would appear to be equally effective in the human.

8. The dog may prove to be a suitable animal for investigating the relation between the type of organism infecting the root canal and the pathology caused. It must be remembered however, that marked variability was observed in the extent of the periapical reaction to inoculation with streptococcus viridans. Seeding canals with similar numbers of bacteria may reduce this variability but this requires further investigation.

Summary

The dog has been evaluated as a suitable animal for endodontic research. The mandibular 2nd, 3rd and 4th premolars are readily accessible for pulp capping, pulpotomy and pulpectomy procedures and the root canals are sufficiently wide to permit instrumentation with standard endodontic equipment. The reaction of periapical tissues to endodontic procedures within the root canal may be studied by sectioning the teeth in situ. Apical perforation is readily achieved and tissue responses to overfilling with various materials may also be observed.

A technique for experimentally infecting root canals and producing apical areas of reaction has been described and this permits studies relating to the efficacy of sterilising methods and the relative value of root canal therapy procedures generally.

The selection of experiments presented in this thesis include the effect of immediate root filling following vital pulpectomy, immediate root filling of experimentally infected canals, the effect of calcium hydroxide on the vital pulp, the use of resorbable pastes, experiments using the corticosteroid/antibiotic preparation Ledermix on vital pulp and in infected canals, and the results of using various paraformaldehyde preparations on pulp and periapical tissue. In each section, a review of the literature has been appended.

A method for handling the animal has been outlined, including the techniques for anaesthesia, radiography, the maintainence of asepsis, perfusion and histological preparation of the material.

The general finding from the series of pilot experiments reported is that dog pulp is more sensitive to trauma and more prone to degeneration than the human pulp and consequently experiences greater difficulty in healing. This is considered of importance when endeavouring to assess the relative tissue compatibility of endodontic materials. There is no evidence that the periapical tissues in the dog or human react in a significantly different manner to endodontic procedures.

The framework of experimentation described in this thesis highlights areas for more definitive study using the dog as the experimental animal.

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