THE ROLE OF THE BLOOD CLOT IN ENDODONTIC THERAPY

AN EXPERIMENTAL HISTOLOGIC STUDY

by

B. NYGAARD ÖSTBY

INTRODUCTION

In general pathology and in surgery the significance of blood and the blood clot has been recognized (*Lorin-Epstein* 1927, *Fränkel* 1929, 1931, and 1932, *Carrel* 1930, and *Allgöwer* 1949). In the healing of bone fractures the blood clot is considered an extremely important factor (*Weinmann & Sicher* 1955, and *Ackerman* 1959). Therefore, it seems strange that in endodontic treatment bleeding is more or less looked upon as a complication to be feared. The writer, for one, has earlier (1958) maintained that a root filling should never be carried out if there are signs of even a slight bleeding in the canal.

However, in an experimental study on the effect of EDTA (*Nygaard Östby* 1957) a case was observed, which suggested that this concept needed re-evaluation.

It was decided to study how the periodontal tissue would react if the entire pulp was removed from the main canal and the apical part subsequently allowed to fill with blood. The aim was primarily to see if the results would have any significance in clinical endodontics. At the same time one might expect that an experimental series planned in this way would reveal details of general interest with regard to the organization of a blood clot. When the latter has connection with live tissue at a small well defined border only, it should offer possibilities for a histologic study of the dynamics of the organization processes. Finally, the purpose of the investigations was to test the effect of EDTAC on the periapical tissues.

MATERIAL AND METHODS

The investigations were carried out on animals and human beings:

1. In three healthy dogs, 8, 8½, and 11 months of age, 8 teeth were extracted under Nembutal anesthesia. The apices were cut off with a diamond disc, whereupon the teeth were immediately replanted. After re-insertion of the tooth, the pulp chamber was opened and the pulp removed. No medicaments were employed and no effort was made to work aseptically. After pulp removal, the cervical part of the canal was plugged with a guttapercha point, coated with a paste made from Kloroperka N-Ö (composition to be described later). The experiments were performed on a minor part of a material used in a study on replantation of teeth (*Löe & Waerhaug* 1961).*)

After observation periods of 42, 51, 60, 70, and 1019 days, the jaws were taken out, fixed in 10 % neutral formalin, and decalcified in 5.2 % nitric acid. They were then cut in proper pieces, which were embedded in paraffin and sectioned in series parallel to the frontal, respectively sagittal plane. The sections were stained with hematoxylin-eosin or with *Goldner's* (1938) modification of *Masson's* connective tissue staining.

2. In 9 human beings, their age varying from 21 to 42 years, 17 teeth were treated. The diagnoses were either clinically intact pulp, open chronic pulpitis, partial necrosis of the pulp, or pulp necrosis with a periapical radiolucent area.

Prior to a description of the treatment, mention will be made of four of the employed agents, viz., EDTAC, a formaldehyde solution, Kloroperka N-Ö, and the culture medium.

a) The chemical, EDTAC, represents a further development of the EDTA solution previously suggested by the writer for use in endodontic treatment (1957), and also used in some cases in the present work. Its formula is as follows:

EDTA (di-sodium salt of ethylenediamine tetra-acetic acid)	143.00 g
CETAVLON® (Cetyl-trimethyl-ammonium bromide)	0.84 g

*) Acknowledgement is given to *H. Löe*, Research Associate at the Norwegian Institute of Dental Research, for his co-operation.

NaOH	q.s.	
Distilled water	ad	1 litre.

The pH of the ready made solution should be 7.4. — The pH of EDTA in aqueous solution varies considerably, and so does, consequently, the quantity of NaOH required. (Usually 10—15 g solid NaOH is required per litre ready made solution). The quantity of EDTA is given as waterfree salt (mol.v. 336.1).

The solution is a chelating agent which presumably effects a superficial demineralization of the dentin, thus facilitating the instrumental cleansing and widening of the root canal. The quarternary ammonium compound, CETAVLON®, has been added to render the solution bactericidal and to lower its surface tension.

b) For the sterilization of the root canals in the necrotic cases a 4 % formaldehyde solution was employed according to the following formula:

40 % formalin	10	cc
Aqua dest.	90	cc
$NaH_2PO_4 \cdot 2\ H_2O$	0.4	g
$Na_2HPO_4 \cdot 2\ H_2O$	0.828	g

c) Kloroperka N-Ö is a powder manufactured according to the following formula:

Balsamum canadense	19.6 %
Resina colophonii	11.8 %
Guttapercha alba	19.6 %
Zinci oxydum	49.0 %

When the powder is mixed with chloroform, a paste is formed, which sets as the chloroform evaporates. The paste is intended for use in conjunction, preferably, with guttapercha points, and will adhere to these and to the root canal walls even if the canal is filled with blood. The setting also takes place uninfluenced by the presence of blood or serous exudate. Previous investigations seem to indicate that the hardened paste is tolerated by pulp and periodontal tissues as a neutral foreign body (*Nygaard Östby* 1944, 1957).

d) All cases except one were tested bacteriologically. The following culture medium was employed:

Brain heart infusion (dehydrated)	DIFCO	18.5	g
Bacto-agar	,,	1.0	g
L-Cysteine		0.5	g
Yeast extract		1.5	g
Hydrosulfite medium*)		50.0	ml
Water		500.0	ml

*) *Hydrosulfite medium:*

Beef extract	DIFCO	1.5	g
Peptone		7.5	g
NaCl		2.25	g
Bacto-agar	DIFCO	0.5	g
Yeast extract	,,	3.75	g
Bacto-dextrose	,,	2.75	g
Sodiumhydrosulfite ($Na_2S_2O_4 \cdot 2\,H_2O$)		0.5	g
or $Na_2S_2O_4$		0.4	g
Methylene blue		0.001	ml

(To 500 ml of medium add 1 ml of a 0.1 % solution of methylene blue).

In all vital cases the treatment was carried out in one sitting under strict asepsis. The pulp chamber was first cleansed and swabbed with 30 % hydrogen peroxide, and subsequently by a solution of 1 % benzalconium chloride in 70 % alcohol. When the pulp chamber was clean and dry, the pulp extirpation was started with Hedström files. The apical part of the pulp was transferred to the culture medium immediately and incubated, and *the result of the culturing was negative in all cases.* Then the root canal was cleansed meticulously to the foramen while flooded with EDTAC, which was renewed every five minutes by means of a pipette. To ensure a profuse bleeding, and also in order to test the effect of EDTAC on the periapical tissues, the files were pushed beyond the foramen and into the alveolar bone. A guttapercha point coated with paste made from Kloroperka N-Ö was then inserted into the canal, while care was taken to leave the apical part unfilled by the material. It should be pointed out that the paste flows easily into the canal and may be pushed farther than the point.

As it is much less radiopaque than the latter, an apparent discrepancy between the roentgenograms and the sections with regard to the extent of the root filling may be encountered.

In three cases a *partial pulp extirpation* was performed in order to compare the results of the two different procedures. The treatment differed only in that respect that in the three cases endeavor was made to leave the apical part of the pulp *in situ.*

In the necrotic cases the debridement was carried out in two sittings. Between the sittings the entire canal contained a suspension of Sulfathiazole® in Lucosil®, introduced with a Lentulo needle in the first sitting after cleansing to the foramen. For medication a 4 % formaldehyde solution was used on a paper point sealed in the canal for some days. The filling of the canal was carried out in exactly the same way as was described for the vital cases, but — with the exception of one case — not until the bacteriologic tests permitted the assumption that the canal had been rendered sterile. The tests comprised:

1) culturing of the deposited paper point originally impregnated with formaldehyde solution, whose bactericidal effect is exhausted after some days,

2) culturing of a paper point inserted to the foramen and left *in situ* for some minutes,

3) culturing of scrapings from the root canal walls in the foraminal part of the canal.

The observation periods were arbitrarily chosen to vary from 13 days to 3½ years, and there were no clinical symptoms in any of the cases during the observation time. The specimens for examination were obtained by surgical removal of the apices with their surrounding structures, according to the writer's method (1939, 1944, and 1957). In some cases, where the surgical removal for various reasons could not be performed, the teeth were extracted for examination. The specimens were fixed in 10 % neutral formalin, decalcified in 5.2 % nitric acid and embedded either in Parlodion or paraffin. After serial sectioning, the staining was carried out with hematoxylin-eosin or with connective tissue staining according to *Masson* and modified by *Goldner.*

RESULTS

Findings in the animal autopsy material

It was established that all the root canals contained live tissue. The extent of this tissue, as well as its structure, varied considerably from case to case.

The length, measured from what was considered the actual foramen, ranged from 300 microns (Fig. 7) to 5 millimeters (Figs. 5 and 6). As to the structure, one case showed pure granulation tissue (Fig. 3), while in others there seemed to be a tendency to a transformation into fibrous tissue (Figs. 2, 4, 7, and 8). Common for all these cases was the presence of necrotic tissue remnants in the root canal, obviously due to a faulty debridement of the canal after the pulp extirpation. The granulation tissue contained numerous capillaries from which sprouts were branching off, and close to the endothelium, undifferentiated perivascular cells were observed (Fig. 4 CD). Some of the capillaries seemed to be open to the empty space in the canal and extravasated erythrocytes were observed (Figs. 2, 3, and 5 BD).

In the rest of the cases the root canal contained in its apical part either fibrous connective tissue (Figs. 1 and 6) or one resembling pulp tissue submitted to an inadequate fixation (Fig. 5).

All the root ends showed signs of resorption having taken place after the replantation. The result of this varied considerably in the different cases from just a rounding off (Figs. 1, 2, 4, 7, and 8) to a removal of nearly the entire root end, also by resorption from within the canal. The edge-like formations created by these processes were embedded in a normal fibrous tissue (Figs. 3 and 6). Healing and repair had occurred in the apical periodontium of those teeth which contained organized tissue (Figs. 1, 5, and 6). Their periodontal membranes comprised thick, more or less functionally oriented fibre bundles, and no inflammatory manifestations. Deposition of cementum on the resorbed root ends was observed, and in one case a new apex, not to be distinguished from a normal one, had been formed (Fig. 5). Cementum had also been deposited on the root canal walls (Figs. 5 and 6), and even bone was found in a canal (Fig. 6).

In two of the cases where the periapical tissue showed normal conditions, there was a severe chronic inflammation 3—5 millimeters from the apex with resorption processes in the root surface (Figs. 1 and 5). In another case a cyst extended along the root surrounded by a zone of intense inflammation, and yet, 4 millimeters from the apex normal conditions prevailed (Fig. 8).

Findings in the human biopsy material

An over-all examination of the material gave the impression that the sequence of the ensuing processes was independent of the state of the original content of the root canal, provided that the debridement had been successful, sterility of the root canal obtained, and a bleeding had been produced prior to the insertion of the root filling material.

Consequently, a division of the cases into groups according to the diagnoses was considered superfluous. On the other hand, even though the processes seemed to follow a certain pattern, there was no strict correlation between their progress and the duration of the observation time. Nevertheless, for practical reasons the material was arranged in accordance with the length of the observation periods.

First of all, it was found that the injury suffered by the periodontal tissues through the manipulation of the root canal files healed in a short time. After 13 days there was an inflammation in the periodontium around a blood clot (Fig. 9 AB), but after 35 days the periodontal membrane above the apex showed complete healing with the exception of one case (Fig. 23), where a small area just outside the foramen seemed somewhat fluid-filled and infiltrated, mainly with plasma cells. Generally, the periodontal membrane remained for some time abnormally wide, due to a slow regeneration of the alveolar bone. Such a regeneration was observed still taking place after 10 months indicated by broad deposits of immature bone with osteoid zones (Fig. 17). The endothelialized cavity in and above the foramen is similar to what has been described in the vascularization of thrombi, where vascular endothelium has been observed enclosing large masses of thrombus to form lacunar channels, by which the circulation is restored more rapidly than by normal organization (*Dible* 1958).

Resorption of the root surface as a result of the reactive inflammation in the apical periodontium was regularly observed, and the repair of this seemed to proceed slowly. In some cases, there was little or no indication of a deposition of cementum (Figs. 16, 17, and 21). The denuded root ends were lined by normal fibrous tissue with the bundles running parallel to their surfaces.

A study of the content of the root canal revealed that the clot, i.e., the fibrin, was gradually replaced by granulation tissue, which in its turn was gradually transformed into fibrous connective tissue. By gradually it is meant that both processes seemed to start at the foramen and proceed into the canal. This impression derived from the cases where the content did not comprise solely fibrous connective tissue. There was a great difference in the structure of the tissue found in the root in the different areas. It was seen already after 35 days (Fig. 9 E) that the foraminal part of the canal contained collagen fibre bundles, even if a small island of fibrin remained surrounded by granulation tissue. Farther down in the canal a fibrin mass was partly surrounded by granulation tissue. This fibrin contained free cells only in the outer zone.

Two other cases gave an even better illustration of the differences in the structure of the canal content (Figs. 10 ABC and 19 ABC). The apical part comprised a well differentiated connective tissue with fibre bundles running parallel to the canal walls. This part had a normal supply of blood vessels and an even scattering of lymphocytes and macrophages. Next to this area a granulation tissue was found, containing a vast number of capillaries, often dilated and with swollen nuclei of the endothelial cells, capillary sprouts, neutrophilic leukocytes, lymphocytes, plasma cells, and macrophages of varying size. Fibroblasts were also present in great numbers, some of them with a small, spindle-formed, darkly stained nucleus, others with a large, lightly stained nucleus, in which the nucleoli were easily discerned. Collagen fibres were delicate and scarce. Close to the capillary walls, cells were observed which had the appearance of undifferentiated, mesenchymal elements (Fig. 10 C).

The next area found was a dense fibrin mass with no blood vessels and infiltrated only by two types of cells: neutrophilic leukocytes and fibroblasts. It was sometimes difficult to differentiate between the two types because the fibroblasts had small, darkly stained, spindle-shaped nuclei, and the nuclei of the leukocytes had adapted themselves to the spaces in the fibrin, were drawn out, and had thus lost their usual form. However, by a close study of the sections, it was observed that the leukocyte nuclei had retained their irregular contour and their rounded ends, while the fibroblasts had nuclei with pointed ends and a regular periphery.

A further development of the processes was found in two other cases (Figs. 11 and 12). The formation of fibre bundles had proceeded farther down into the canal and the organization of the fibrin seemed almost accomplished. In one case only a small area persisted in the middle, while a fibrous tissue was found on both sides along the walls with blood vessels and fine collagen fibres (Fig. 11). One capillary was open to the seemingly empty canal, and around the opening and in the canal erythrocytes were found, indicating a recent bleeding. In the other case (Fig. 12) rests of the fibrin seemed to persist in a cell-free narrow band stained red in hematoxylin-eosin and dark green in Masson, with a sharp border against a differently stained band, representing the outer zone of the root filling material. Delicate collagen fibres and capillaries with erythrocytes were found close to the fibrin zone.

In several cases the transformation into fibrous connective tissue seemed completed (Figs. 13, 14, 16, 17, 18, and 23). Sometimes it could be observed ending in a capsule, in which the fibre bundles were arranged parallel to the apical surface of the root filling material (Figs. 13 B, 16, and 23 B). In one case (Fig. 18 AC) it seemed to end in a deposition of cellular cementum, which together with a darkly stained mass including dentin fragments occluded the canal. In another, it ended in a fibrous capsule, the fibre bundles of which were running across the canal above an empty space (Fig. 21).

The described processes in the canal were accompanied by changes in the surface of the canal walls. The formation of granulation tissue was always observed to result in a resorption of the surrounding walls (Figs. 9 E, 10 D, 11, 12, 17, and 19). The resorption process was usually succeeded by a deposition of cementum as the transformation into fibrous tissue took place, even if the tissue still showed signs of inflammation (Figs. 19 and 23). Usually, the product of this activity had the appearance of cellular cementum, which could be of considerable thickness (Figs. 13, 18, and 23). Only in one case (Fig. 14) an acellular cementum lined the canal walls, and the fibre bundles which run across the canal, had been inserted into the deposition. Cementum could, however, be totally absent in the canal, even if this contained normal fibrous tissue (Fig. 17).

The accessory canals encountered in the sections, contained fibrous tissue, even in a root which contained a necrotic pulp before the treatment (Fig. 21). They were twisted and it was, therefore, difficult to examine their entire length and, especially, to find their orifice. However, it was possible in two cases to observe that there was vital tissue in the orifice, cell-infiltrated a short time after the removal of the tissue in the main canal (Fig. 10 F) and lined by fine fibre bundles where the observation period was longer (Fig. 19 D).

Examination of the cases where a *partial pulp extirpation* had been endeavored, revealed that the apical part of the canal contained normal vascularized fibrous connective tissue. In the case with 35 days observation time (Fig. 9 D) such a structure was found above a fibrin clot completely devoid of cell elements, and between walls, which had been extensively resorbed. At the foramen an active deposition of cementum was observed. The two other cases (Figs. 13 C and 18 DEFG) showed a fine fibrous structure and broad deposits of cellular cementum on resorption lines in the dentin. Hard tissue of similar appearance occluded the canal coronally to the soft tissue and above darkly stained masses with dentin fragments.

There were three cases in this biopsy material which showed conditions completely different from the rest, and which, therefore, will be dealt with separately (Figs. 15, 20, and 22). In the first case, the canal was observed to contain a granulation tissue with only traces of collagen fibres. There was an abundance of blood vessels filled with erythrocytes, monocytes, and lymphocytes (Fig. 15 C). In the loose ground substance lymphocytes, plasma cells, and macrophages were found, as well as fibroblasts, the cytoplasm of the latter being clearly discernible (Fig. 15 D).

The picture was dominated by rows of epithelial cells, which had actually formed a membrane around the top of the root filling material (Fig. 15 B).

In the next case the roentgenograms showed no indication of a regeneration in the apical periodontium (Fig. 20). The short space in the root canal above the root filling contained a structureless tissue infiltrated by lymphocytes and macrophages and completely devoid of blood vessels and collagen fibres.

The third case (Fig. 22) showed a root canal filled with necrotic pulp tissue containing some dentin fragments, and an abscess outside the foramen. Fibre bundles had formed a broad capsule around the apex, with accumulations of inflammatory cells in the spaces between the bundles. These were surrounded by a thin continuous bone capsule traversed by blood vessels.

The employed root filling material was in some cases found in contact with vital tissue in the canal, and it was observed that when the organization of the blood clot had been completed, a fibrous capsule had been formed, in which the bundles were running parallel to, and in contact with the surface of the root filling material (Figs. 13 B, 16, and 23).

Hardened Kloroperka N-Ö paste was encountered in the preexisting tissue of an accessory canal, and in the apical periodontium. The small pieces were surrounded by fibrous tissue, which sometimes contained giant cells in contact with the material (Fig. 19 EG), but deposition of bone onto the material could also be observed (Fig. 19 FH). In one case the paste had obviously fastened to the root canal wall and to the root surface outside foramen (Fig. 10 E).

DISCUSSION

The extension of periodontal tissue into the root canal has been reported by many investigators (*Tollardo* 1931, *Honegger* 1932, *Biolcati & al.* 1942, *Nygaard Östby* 1944, *Engel* 1950, *Kukidome* 1957, *Hyakusoki* 1959, *Kuroiwa* 1960, *Matsumiya & Kitamura* 1960). The phenomenon has mainly been shown to take place in cases where the canal had been filled with a resorbable material after complete pulp removal.

The dynamics of such a process have not hitherto been comprehensively studied, neither has the role of a blood clot in the root canal in these cases been considered. It is conceivable that the filling procedure sometimes may cause a rupture of the capillaries in the granulation tissue just outside the foramen, with a following seepage of blood into the apical part of the canal.

In the present investigation there can be no doubt that a blood clot had been formed in the apical periodontium and in the root canal after the treatment. In the dogs' teeth, which were reinserted into a blood-filled socket, the extent of the clot depended on seepage and capillary traction of the blood into the canal, and consequently was impossible to assess. The canals of the human teeth, with the one exception where no bleeding was produced, were filled with blood. A blood clot must, therefore, subsequently have occupied the entire space left open after the treatment.

The fact that the lacerated periapical tissues healed in a short time is not surprising, as this process can be directly compared with that taking place in bone fractures. If anything should be noted as remarkable in this connection, it would be that the periodontal membrane was restored. In no instance a union between the root and the alveolar bone was observed, not even in the animal material, where the entire continuity had been severed. While the structure of the periodontal membrane was quickly restored, and the alveolar bone seemed to regenerate within a reasonable time, the deposition of cementum onto the denuded root surface failed to take place in some cases. A similar observation has been made in previous investigations (*Nygaard Östby* 1939 and 1944), and the present findings corroborate what earlier has been maintained by the writer: A denuded, ragged root end *does not* in itself represent an irritating foreign body, and *does not* exert a chronic irritation which impedes a healing in the apical periodontium.

Another notable observation was that the laceration of the periapical granulation tissue in the necrotic cases seemed to stimulate the healing. This impression derives from only three cases, but they are from the same individual and should, therefore, be directly comparable (Figs. 16, 20, and 21). This observation seems to be in accordance with others made within general pathology. *Lorin-Epstein* (1927) and *Fränkel* (1929, 1931, and

1932) studied the "necrohormones" emanating from injured tissue and considered them significant in the healing of bone fractures, and *Carrel* (1930) maintained that growth-activating polypeptides may be manufactured by leucytic ferments from cell debris and coagulated fibrin. *Allgöwer* (1949), who succeeded in growing infected human tissue in a plasma coagulum with Sulfathiazole *in vitro,* reported striking results from treating torpid, infected wounds by creating a blood clot which filled all the recesses. He purported that the components of the blood stimulated the vitality of the granulation tissue.

When the findings in the root canals are to be discussed, it must be kept in mind that only in the animal experiments, where the tissue continuity was definitely severed, can one be sure that no vital tissue remained in the canal. In the human teeth this could not be ascertained, and there is always the possibility that some pulp remnants had been left along the root canal walls, and that they had remained vital by retaining their connection with the periodontal membrane, possibly through accessory canals. Remaining vital strands of the pulp may have participated in the organization of the blood clot, while, on the other hand, necrotic pulp tissue remnants left in the canal seemed to impede this process.

The extent of the organization process into the root canal seems to be limited, as even in the cases where the process was not stopped by the root filling material, the tissue plug terminated at a certain distance from the foramen, and left an open space in the root canal. In the human specimens, at least, it must be presumed that this space originally was filled with blood, and in the sections, remnants of fibrin can be found along the root canal walls and also in the middle of the canal. It is inconceivable that the open spaces in the canal are artifacts, and it is, therefore, more likely to ascribe the phenomenon to a desiccation of the fibrin clot at a certain distance from the foramen. *Kovac & Rudas* (1960), carrying out experimental investigations on the dynamics of the granulation tissue, observed similar conditions in their material. They found it remarkable that a wound, which is not epithelialized, produces a termination against the surface when the scab, on account of desiccation and the ensuing crumbling, does not form an adequate protection. In their material the newly formed granulation tissue was covered by a thick layer of collagen with vessels, which obviously protected the underlying tissue against desiccation. Similar observations were made in the present material, which also corroborate the mentioned authors' statement that granulation tissue is transformed into collagen without the presence of epithelium. *Kovac & Rudas* also observed that the new capillaries grow into the empty space in the way that their growth pole is surrounded by a small bleeding. This means that when the capillary sprouts undergo a hollowing, blood will be discharged from their openings into the empty space. The same phenomenon was observed in the present material, and this is the explanation why sometimes a spontaneous bleeding is encountered during root canal treatment.

In the cases where the termination of the tissue plug in the root canal consisted completely or partly of fibrin, this mass was infiltrated only by two different cell elements, namely neutrophilic leukocytes and fibroblasts. The observation that the nucleus of the neutrophilic leukocytes adapts itself to the spaces in the fibrin mass, thus getting a drawn out, almost spindle-formed shape, was already made by *Aschoff* (1892), who found these forms in the thrombus, but not in the "red blood". *Langeland* (1957) made the same observation in dentinal tubules into which neutrophiles had migrated. The finding of spindle-shaped fibroblast nuclei in wound coagula is also reported by *Allgöwer* (1956), who considered this a confirmation of the probable relationship between blood cells as the source and these elements as the product. In the present material the fibroblasts in the fibrin clot may just as well be assumed to have migrated from the adjacent granulation tissue, as described in studies on the development of connective tissue in transparent chambers (*Stearns* 1940). In these studies it was observed that fibroblasts from the surrounding tissue migrated into an experimentally created wound in a very short time, and that they started to form collagen when the ingrowing capillaries had reached a certain distance from them.

It is generally agreed that capillaries normally are surrounded by loose connective tissue containing, among other elements, undifferentiated cells with mesenchymal potentialities. The question whether this is valid also for capillaries, which have been formed in a granulation tissue, i.e., extensions of pre-existing vessels, has been discussed, a.o., by *Werthemann* (1928). He concluded that the newly formed capillaries were embedded in a "network of fibrocytes" and that this had the same potentialities as a mesenchymal, multipotent, undifferentiated tissue. The observation in the present material of perivascular elements, resembling undifferentiated mesenchymal cells, close to the capillaries, tends to support this view. As these cells can differentiate into osteoblasts and cementoblasts, the formations of the different hard tissues in the root canals may thus be satisfactorily explained.

It has been maintained (*Kovac & Rudas* 1960) that a bleeding is not necessary as the primary wound covering, and that an organization can take place in a coagulated exudate. When the findings in the present material is compared with those in earlier works by the writer (1939 and 1944), this does not seem to be valid in the root canals. In the cases where the canal had been filled in such a way that there were open spaces in the apical part, and no bleeding had been produced prior to the root filling, the spaces have invariably been found to contain exudate, infiltrated by vital and dead neutrophilic leukocytes and no organized tissue, even after an observation time of several years. On the other hand, it is seen in the present material that when a bleeding was created, the space in the canal contained a more or less organized tissue. In the case where there was no bleeding prior to the root filling, the canal contained only an accumulation of cells and neither capillaries nor collagen. Consequently, the blood and the blood clot seem to be essential for the formation of fibrous connective tissue in an empty root canal.

Clinical aspects

It is self-evident that no far-reaching clinical inferences can be made from the limited material presented, neither does the writer intend to suggest a new endodontic rationale on the basis of his findings. On the other hand, the establishment of the fact that a blood clot in the root canal is organized to a certain extent, and that the ultimate result of this process under certain conditions is a normal fibrous tissue, may be instrumental in changing the view on the therapeutic problems within clinical endodontics. In the following, the results from the experiments will be used to explain certain phenomena, and also to intimate a future development of endodontic therapy.

It was mentioned earlier that vital tissue had been observed in root canals previously subjected to a complete debridement and filled with a resorbable material, and that this phenomenon could be explained as a result of a bleeding and a subsequent organization of the blood clot. A similar process may take place when other kinds of root filling materials are used. Fig. 24 shows a case, in which the canal has been overfilled with guttapercha and Kloroperka N-Ö paste. Eighteen years later the excess had disappeared, and the foraminal part of the canal contained fibrous tissue, which had deposited secondary cementum on the wall almost to a closure of the canal. It is possible that the material did not fill out the foraminal part completely, and that the overfilling had created a bleeding, resulting in a blood clot in unfilled spaces in the canal. During the organization of the clot, the material in this part may have crumbled and been transported away by cells.

Usually the two materials, guttapercha and the hardened Kloroperka paste, have been found surrounded by a fibrous capsule in the periapical tissue (*Nygaard Östby* 1939, 1944, and 1957, *Coolidge & Kesel* 1956). During the regeneration, bone is formed almost in contact with the materials, and even onto the Kloroperka. In this way the latter may be superimposed by the regenerated alveolar bone in the follow-up roentgenograms, as the radiopacity is about the same. It is very doubtful that it is actually resorbed or absorbed *sensu strictori*. The so-called resorbable materials disappear completely after some time, as can be ascertained in the sections (*Nygaard Östby* 1939 and 1944, and *Engel* 1950), either by being dissolved in the tissue fluids or broken down and digested by free cells. As to guttapercha and Kloroperka, *Coolidge & Kesel* (1956) and *Nygaard Östby* (unpublished investigations) have observed particles of these materials in the cytoplasm of macrophages after a long observation time.

In the present study multinucleated foreign body giant cells are found in contact with the hardened Kloroperka paste. These specific cells are formed just around insoluble materials, as for instance non-absorbable sutures. Even if these cells generally are considered not to be very active, they must — considering their origin — be attributed a certain motility. It is conceivable that small non-resorbable particles can be transported from the canal and the vicinity of the apex to areas, where they will be superimposed radiographically by the alveolar bone. This pertains to small particles, however, and the disappearance of a gross excess of guttapercha and Kloroperka, sometimes observed, is more difficult to explain. If this occurrence, which by no means is the usual one, were due to a breaking down of the continuous mass by cellular activity, it should be possible to find manifestations of such an activity by histologic investigation of specimens from cases where the materials have been used. However, in the writer's entire collection of histologic sections there have never been observed lacunae with resorbing cells in the surface of the two materials in question. Neither has a spontaneous removal of the same been found to take place, when a *complete* filling of the canal in its cross-section had been accomplished. The case presented in Fig. 24 is illustrating in this respect: at the level in the canal where there is a total closure, the materials are adjoined by a normal fibrous tissue. It is inconceivable that it would have been so, if these materials were susceptible to a cellular resorption.

There is another, if somewhat speculatory, explanation of the occasional disappearance of the excess, namely a crumbling of the materials sufficiently for them to be subjected to the formerly mentioned cell activity. It is a fact that the paste of Kloroperka becomes very brittle, when the chloroform has evaporated, and the same is true for guttapercha, probably due to oxidizing processes. In the mesodermal tissue, especially where a foreign body has been implanted, there is a constant movement of all inherent elements: fluids, fibroblasts, wandering cells, as well as formation of new capillaries, collagen and fibre bundles; in short, an everchanging pattern. When the movements of the apex of the tooth in function is taken into consideration as well, it might be possible to imagine that a crumbling of brittle implants can take place in the apical periodontium.

The partial pulp extirpation, experimentally investigated and recommended by the writer (1939), is still in his opinion the method of choice, and the results of that method in the present work confirm this statement. In order to ascertain clinically that the apical part of the pulp actually has been left *in situ,* it has been recommended by the writer to carry out the treatment in two sittings. Prior to the root filling in the second sitting, the canal is probed with a file to establish sensitivity at the point where the severance of the pulp had been endeavored. Now, with the knowledge that if the pulp accidentally should be torn at the foramen, a blood clot will form in the apical part of the canal, the procedure is tentatively changed in the writer's practice. In easily accessible canals the treatment is carried out in one sitting, and care is taken not to push the root filling material beyond the supposed point of severance.

It sometimes happens that the root canal instruments are pushed too far before the first control roentgenogram is taken, thus lacerating the apical part of the pulp (see Fig. 25). Previously, there seemed to be nothing else to do in these cases than to ream out the entire canal and fill it to the periodontal membrane. It has been shown, however, by the writer (1939 and 1944) that the risk of an overfilling is great under these circumstances, and also that the healing of the apical periodontium is slow in the presence of even an inert foreign body. There should be an alternative now to this procedure, and this appears from the roentgenograms in Fig. 25. Here, the file has just been withdrawn and a partial pulp extirpation then carried out on the basis of the reasoning that the lacerated pulp tissue would participate in the organization of the blood clot.

In the treatment of necrotic pulps, one of the greatest problems is to fill the canal adequately after debridement and sterilization. That this is of decisive importance for the result has been shown beyond doubt both experimentally and clinically. If a deep-going open space, be it ever so narrow, is left between the root filling material and the canal wall, it will be filled with exudate and constitute a permanent source of irritation, preventing a complete healing in the apical periodontium. It is also probable that microorganisms, during a transient bacteremia, may migrate from the capillaries of the granulation tissue just outside foramen and into these spaces.

The present study may contribute to a solution to the problem of an adequate filling of the canal in necrotic cases. As pointed out in the previous part of this chapter, a laceration of the granulation tissue outside foramen does rather seem to have a beneficial effect on the healing process. Furthermore, the blood itself has inherent bactericidal properties and cells which develop into phagocytes. A discharge of blood into the root canal may have two effects: destruction of remaining micro-organisms and phagocytosis of necrotic debris. At last comes the most important achievement: the organization of the blood clot, and formation of fibrous tissue in the apical part of the canal after the root filling. It seems as if this can occur even in very narrow spaces. However, it must be assumed that a wider space is more favorable, because it probably allows a more rapid ingrowth of capillaries promoting the formation of collagen.

While the preceding reflections must be considered an outline for future research, there is one specific treatment problem where, in the writer's opinion, the results of his investigations could be applied clinically already at this stage. The traumatized front tooth with a necrotic pulp in an undeveloped root represents probably the greatest problem and challenge in the field of endodontics. The rationale, suggested in these cases, appears from Fig. 26, which shows a case of a traumatized front tooth with a necrotic pulp and a large radiolucent area above its undeveloped root end. After debridement and sterilization a bleeding was created and the canal plugged in the cervical part. There can be no doubt that the radiolucency has disappeared after a year, and that the root end has a different shape after another four months. It seems as if a development of the apex is taking place, and recalling the findings in the animal material, it is conceivable that this is what actually happens.

It can be discussed whether the root filling should not be placed nearer the foramen. On the other hand, the conditions in these canals may be more favorable than those in the experiments, and, therefore, a more extensive organization may be expected. It has been shown that the process is accompanied by deposition of hard tissue on the root canal wall, and the ideal outcome is, of course, beside the development of the apex, a reduction of the width of the canal.

The present investigations may be said to introduce a biologic view of the treatment problems: the utilization of the growth potentialities of the body. In this connection it is natural to mention the possibility of employing growth promoting substances in the treatment. In general pathology many bio-assays have been carried out especially on local application of tissue extracts (*Edwards & al.* 1960) and vitamin C, and the latter seems to be most suitable for our purpose. The organization of the blood clot in the apical periodontium and in the root canal may be directly compared with wound healing. It has been established unequivocally that the failure of wounds to heal normally is due primarily to the failure of the body to produce collagen, and in animal experiments it has been clearly shown that there is a direct relationship between ascorbic acid intake and the healing of wounds. *Woessner & Gould* (1957) proposed the hypothesis that ascorbic acid may not play a direct role in slow collagen synthesis such as normally occurs, but that it may do so in rapid collagen synthesis such as is encountered in wound healing, and *Gould & al.* (1960) have presented evidence to substantiate the idea that ascorbic acid is essential for the maintenance of newly formed collagen and of blood vessel walls.

This pertains primarily to the general state of the body with regard to vitamin C, and indicates the importance of an optimal level during and after endodontic treatment. Besides, however, there is evidence that local application of ascorbic acid has a beneficial effect on the healing process. *Saitta* (1929) was probably the first who claimed to have observed an increase in the rate of healing when vitamin C extracts were applied to the surface of wounds of animals on normal or scorbutigenic diets. *Gould* (1960) maintains that he has "presented evidence for a direct specific effect for ascorbic acid in collagen biosynthesis *in vivo*." He has employed quantitative methods based on hydroxyproline synthesis as a measure of collagen formation in a comprehensive experimental study of the effect of local application of vitamin C. Paired polyvinyl sponge implants were made subcutaneously, and the administration of relatively small doses of ascorbic acid into one of them resulted in more collagen formation in this than in the saline-injected control sponge in the same animal.

The results of these experiments might have a direct bearing on endodontic treatment in the future. In further investigations along the lines suggested earlier, it might be advantageous also to consider the modern research on collagen formation, as well as that on hard tissue forming cells: the osteoblast, the cementoblast, and the odontoblast. It appears that these cells also are influenced by ascorbic acid deficiency, and *Follis* (1951) has characterized such deficiency by a failure of the cells to promote the deposition of their respective fibrous protein: osteoid, cementoid, and predentin.

The present investigation was also intended to be a histologic bio-assay of the chelation agent EDTAC. In a previous study (*Nygaard Östby* 1957) it has been shown that a 15 % solution of EDTA at a neutral pH has a demineralizing effect on the dentin. Furthermore, the preparation seemed to be innocuous to pulpal and periodontal tissues. The Cetyl-trimethyl-ammonium bromide was added to the solution on the presumption that this would enhance its cleansing effect and render it bactericidal. Even if the addition clinically seemed neither to alter the demineralizing effect nor to produce any untoward reactions in the soft tissues, it was felt that the new preparation should be subjected to a similar test as the first one.

First it was ascertained by chemical analysis that the quarternary ammonium compound could be recovered from the solution after several years, which means that the two chemicals do not react with each other.

Then the demineralizing effect of the new preparation on the dentin of the root canal walls was investigated by means of microradiography. These investigations, which will be dealt with in a future publication, showed that already after 5 minutes there was a superficial demineralization of the root canal wall.*) Moreover, it was established that the preparation was self-limiting,

*) In a recently published work the dentin dissolving capacity of EDTA was compared with that of 50% sulphuric acid. It was established that the former is significantly more effective. (*Ramström* 1961).

which corroborates the statement made earlier by the writer (1957) with regard to EDTA.

In the discussion of the influence of EDTAC on the soft tissues, some specific aspects must be taken into consideration. First of all, it acts as an anticoagulant, because it chelates calcium from the blood, thus interfering with the clotting process (*Godal* 1960). If it is injected subcutaneously in animals, the hemorrhage will be extensive and, consequently, the reactions severe in the surrounding tissues. Unfortunately, this is not always realized, and erroneous conclusions have been drawn from experiments of this kind (*Patterson* 1960).

When the solution is applied in the root canal, it acts primarily on the dentin. However, it is self-evident that the calcium chelated from the latter will not render the solution completely inactive in the short time before it comes in contact with the soft tissue. Consequently, the clotting of the blood will be delayed and calcium will be subtracted from the tissue fluid. Clinically, the effect is insignificant, and histologically, it is impossible to establish any manifest differences between the cases in which EDTAC has been used during the treatment and those of the writer's earlier investigations.

The relative compatibility of EDTAC has also been shown by *Torneck* (1961), who tested ten various drugs commonly used in endodontic treatment. In these experimental investigations, carried out subcutaneously in hamsters, an aqueous solution of potassium penicillin G and EDTAC produced the least tissue reaction of all the drugs tested. The reaction outside the punctures in the polyethylene tube containing EDTAC was similar to that found in the controls where physiologic saline was used.

SUMMARY

In order to study the consequences of a bleeding from the periapical tissues and the formation of a blood clot in the root canal, a series of experimental investigations was carried out on dogs and human beings. A bio-assay of the chelating agent EDTAC, suggested by the writer as an adjuvant in endodontic therapy, was included.

In the animals, intact teeth were extracted, the apex cut off, and after immediate replantation, the pulp was removed through the crown. In the human teeth, with diagnoses ranging from "clinically intact pulp" to "complete pulp necrosis", the content of the main canal was removed and prior to the insertion of the root filling material, a bleeding was produced by lacerating the periapical tissues through the foramen. In all the teeth, the canal was partly filled with guttapercha points coated with a paste made of Kloroperka N-Ö powder in chloroform, allowing a blood clot to be formed in the apical portion.

After observation periods, ranging from 13 days to 3½ years, the animal autopsy material and the human biopsy material were submitted to a histologic examination. The results from the writer's earlier experimental investigations served as controls.

It was observed that the blood clot in the root canal was organized probably by granulation tissue growing in from the periapical area and not from the blood cells originally contained in the clot. Even when it was not limited by the root filling, the organization did not proceed far into the canal, but terminated at a distance from the foramen. The phenomenon is ascribed to a desiccation and crumbling of the rest of the fibrin, like the scab in a wound. When the ingrowing tissue reached this level in the canal, the matrix for a further organization had deteriorated. The granulation tissue was gradually transformed into fibrous connective tissue, provided that the original content of the root canal had been carefully removed, and that the canal was sterile. Such a transformation even took place in cases where the diagnosis had been pulp necrosis with infection and apical periodontitis. The fibrous tissue formed could not be distinguished from that found after a partial pulp extirpation. In many cases it had deposited cellular cementum on the root canal walls, which usually showed signs of resorption having occurred during the organization of the clot.

The writer does not intend to suggest a new endodontic rationale. Even though the material may be too limited for him to do so, the findings seem to have a few clinical implications, which have made the writer tentatively modify some of his rigid treatment principles. The most important modification pertains to the therapy in cases of pulp necrosis in teeth with an undeveloped apex. Instead of trying to fill out completely the entire root canal after debridement and sterilization, the method described in this work is employed. The clinical results have been encouraging in the respect that a further subsequent development of the apex seems to have taken place, as judged from the follow-up roentgenograms.

With regard to the agent EDTAC, the findings gave no evidence that its application in the root canal and in the apical periodontium had done any irreversible harm to the tissues or interfered with the healing processes to any appreciable extent.

RÉSUMÉ

LE RÔLE DU CAILLOT DANS LE TRAITEMENT ENDODONTIQUE

Dans le but d'étudier les conséquences d'une hémorragie des tissus périapicaux et la formation d'un caillot sanguin dans le canal radiculaire, une série d'études expérimentales a été effectuée sur des chiens et sur des sujets humains. Cette série comprenait un bio-contrôle de l'agent de chélation EDTAC, proposé par l'auteur comme adjuvant dans la thérapeutique endodontique.

Sur les animaux, des dents intactes, ont été extraites, l'apex réséqué, et, après réimplantation immédiate, la pulpe a été enlevée par voie coronaire. Dans les dents humaines, au diagnostic allant de "pulpe cliniquement intacte" à "nécrose pulpaire", le contenu du canal principal a été enlevé, et avant l'insertion du matériel d'obturation radiculaire, une hémorragie a été provoquée en lacérant les tissus périapicaux à travers le foramen. Dans toutes les dents, le canal a été partiellement obturé avec des cônes de gutta-percha enrobés d'une pâte faite de "Kloroperka N-Ø" dans du chloroforme, permettant la formation d'un caillot sanguin dans la partie apicale.

Après des périodes d'observation allant de 13 jours à 3 ans ½, le matériel d'autopsie animal et le matériel de biopsie humain ont été soumis à un examen histologique. Les résultats des expériences antérieures de l'auteur ont servi comme contrôles.

L'auteur a observé que le caillot sanguin du canal radiculaire

s'était organisé, probablement grâce à des tissus de granulation se développant à partir de la region périapicale, et non grâce à des cellules sanguines contenues à l'origine dans le caillot. Même dans les cas où elle n'était pas limitée par l'obturation radiculaire, cette organisation ne s'étendait pas bien loin dans le canal, mais s'arrêtait à une certaine distance du foramen. Ce phénomène est attribué à une dessiccation et un émiettement du reste de la fibrine, comme la croûte d'une plaie. Quand le tissu qui se développe vers l'intérieur atteint ce niveau dans le canal, l'organisation ne peut se poursuivre, la matrice s'étant détériorée. Le tissu de granulation se transforme graduellement en tissu conjonctif fibreux, à condition que le contenu originel de la racine ait été soigneusement enlevé et que la canal soit stérile. Cette transformation s'est même produite dans des cas où avait été posé le diagnostic de nécrose pulpaire avec infection et d'inflammation périapicale. Le tissu fibreux formé était impossible à distinguer de celui qu'on trouve après une extirpation partielle de la pulpe. Dans bien des cas, il s'était déposé du cément cellulaire sur les parois du canal radiculaire, qui présentaient en général les signes d'une résorption s'étant produite pendant l'organisation du caillot.

L'auteur ne se propose pas d'indiquer une nouvelle méthode endodontique. Bien que le matériel puisse être trop limité, les résultats semblent avoir quelques implications cliniques qui ont poussé l'auteur à modifier à titre d'essai quelques uns des ses fermes principes thérapeutiques. La modification la plus importante concerne le traitement des cas de nécrose pulpaire de dents dont l'apex n'a pas terminé son développement. Au lieu d'essayer d'obturer complètement la totalité du canal radiculaire après nettoyage et stérilisation, l'auteur a utilisé la méthode décrite dans la présente étude. Les résultats cliniques ont été encourageants, en ce sens qu'un développement ultérieur de l'apex semble s'être produit, à en juger par les radiographies de contrôle.

En ce qui concerne l'agent EDTAC, les résultats n'ont pas montré que son application dans le canal radiculaire et dans la region périapicale ait eu sur les tissus une action nuisible irréversible ou entravé le processus de cicatrisation de manière appréciable.

ZUSAMMENFASSUNG

DIE ROLLE DES BLUTKOAGULUMS IN DER WURZELBEHANDLUNG

Um die Folgezustände einer Blutung aus den periapikalen Geweben und die Bildung eines Koagulums im Wurzelkanal abzuklären, wurde an Hunden und Menschen eine Serie von experimentellen Untersuchungen durchgeführt. Zudem wurde das Präparat EDTAC, das vom Verfasser als Adjuvans zur Therapie des Wurzelkanals empfohlen wird, im biologischen Versuch überprüft.

Intakte Hundezähne wurden extrahiert, ihre Wurzelspitzen abgeschnitten, und nach sofortiger Replantation die Pulpa durch die Zahnkrone entfernt. Bei den menschlichen Zähnen, mit unterschiedlichen Diagnosen von „klinisch intakter Pulpa" bis „Pulpanekrose", wurde der Inhalt des Hauptkanals entfernt; durch absichtliche Verletzung der periapikalen Gewebe, vom Wurzelkanal aus, wurde vor dem Legen der Wurzelfüllung eine apikale Blutung hervorgerufen. Bei allen Zähnen wurde der Kanal mit Guttaperchaspitzen, die mit der „Kloroperka-Chloroform-Paste" nach Nygaard Østby überzogen waren, nur partiell abgefüllt, so dass sich im apikalen Teil des Wurzelkanals ein Blutkoagulum bilden konnte.

Nach Beobachtungszeiten zwischen 13 Tagen und 3½ Jahren wurde das tierische Autopsiematerial und das menschliche Biopsiematerial histologisch untersucht. Die Resultate von früheren experimentellen Untersuchungen des Verfassers dienten als Kontrolle.

Es konnte beobachtet werden, dass das Blutgerinsel wahrscheinlich von Granulationsgewebe, das aus dem apikalen Periodontium hineinwucherte, und nicht von den im Koagulum befindlichen freien Blutzellen, organisiert wurde. Der Organisationsvorgang erstreckte sich nicht weit in den Kanal hinein, sondern *machte in einer gewissen Entfernung vom Foramen halt*, auch in denjenigen Fällen, wo die Wurzelfüllung ein weiteres Vordringen des Gewebes nicht behindert hatte. Diese Tatsache wird damit erklärt, dass das Blut in den von der Wundfläche weiter abgelegenen Teilen im Sinne einer Schorfbildung austrocknete. Wenn das einwachsende Granulationsgewebe diesen verschorften Bezirk erreichte, so hörte die weitere Organization auf.

In denjenigen Fällen, wo der Kanal steril und sein ursprünglicher Inhalt vollständig ausgeräumt war, wurde das Granulationsgewebe allmählich in ein fibrilläres Bindegewebe umgewandelt. Diese Umwandlung trat auch in den Fällen auf, wo die Pulpa nekrotisch und infiziert gewesen war und eine apikale Periodontitis vorgelegen hatte. Das im Kanal vorhandene Bindegewebe konnte nicht von jenem Gewebe unterschieden werden, das nach einer partiellen Pulpaexstirpation gefunden wird. In mehreren Fällen war zelluläres Zement auf die Wurzelkanalwände abgelagert, die ihrerseits gewöhnlich deutliche Zeichen von Resorptionen als Folge der im Kanal ablaufenden Organisationsvorgänge aufwiesen.

Der Verfasser beabsichtigt auf Grund dieser Beobachtungen keineswegs neue Behandlungsmethoden vorzuschlagen. Indessen veranlassen ihn die erhobenen Befunde, auch wenn es sich hierbei nur um ein beschränktes Untersuchungsmaterial handelt, gewisse therapeutische Prinzipien versuchsweise zu modifizieren: die wichtigste Modifikation betrifft die Behandlung von pulptoten Zähnen mit nicht abgeschlossenem Wurzelwachstum. Anstelle der vollständigen Wurzelkanalabfüllung (nach Ausräumen und Desinfektion des Kanals) wird die in dieser Arbeit beschriebene Methode verwendet. Die klinischen Resultate waren insofern ermutigend, als das weitere Wurzelwachstum auf Grund der Kontrollröntgenaufnahmen unbehindert vor sich zu gehen scheint.

Hinsichtlich des Präparates EDTAC ergaben die Befunde keinen Hinweis, dass seine Applikation im Wurzelkanal die periapikalen Gewebe nachhaltig schädigen könnte oder die Heilprozesse merkbar beeinflusst hätte.

RESUMEN

PAPEL DEL COÁGULO SANGUÍNEO EN LA TERAPIA ENDODONCICA

Se realizaron una serie de investigaciones experimentales en perros y en humanos con el objeto de estudiar las consecuencias de la hemorragia de los tejidos periapicales y la formación del coágulo sanguíneo en el conducto radicular. Se incluyó en el estudio una doble experiencia del agente quelante EDTAC sugerido por el autor como coadyuvante en la terapia endodóncica.

Se extrajeron dientes intactos de animales, se seccionó el ápice, y después de una reimplantación inmediata se sacó la pulpa a través de la corona. En dientes humanos con diagnóstico que variaba desde "pulpa clínicamente intacta" hasta "pulpa totalmente necrótica" se extrajó el contenido del conducto principal y antes de colocar el material de obturación radicular se provocó *una hemorragia lacerando los tejidos periapicales a través del* foramen. En todos los dientes el conducto fué obturado parcialmente con conos de gutapercha forrados con una pasta formada con polvo de "Kloroperka N-Ö" en cloroformo permitiendo la formación de un coágulo sanguíneo en la porción apical.

Transcurridos períodos de observación que variaron desde 13 días a 3½ años el material de autopsia de animales y el material de biopsia humano fueron sometidos a un examen histológico. Los resultados de investigaciones experimentales realizadas anteriormente por el autor sirvieron como casos testigos.

Se observó que el coágulo sanguíneo en el conducto radicular estaba organizado probablemente por tejido de granulación que había penetrado desde la zona periapical y no por las células sanguíneas contenidas originariamente en el coágulo. Aún cuando no estaba limitado por la obturación, el tejido de granulación no avanzaba mucho dentro del conductó, sino que terminaba a cierta distancia del foramen. El fenómeno es atribuído a una desecación y destrucción del resto de la fibrina como la costra en una herida. Cuando el tejido que proliferaba hacia adentro alcanzó este nivel en el conducto, la matriz, por una posterior organización, se había deteriorado. El tejido de granulación se transformó gradualmente en tejido conjuntivo fibroso siempre que el contenido original de la raíz se hubiera extirpado cuidadosamente y que el conducto fuera estéril. Tal transformación tuvo lugar aún en los casos con diagnóstico de necrosis pulpar con infección y *periodontitis apical. El tejido fibroso formado no podía distinguirse* del encontrado después de una extirpación pulpar parcial. En muchos casos se había depositado cemento celular sobre las paredes del conducto, las cuales usualmente mostraron signos de reabsorción producida durante la organización del coágulo.

El autor no intenta sugerir un nuevo fundamento o método de endodoncia. Aún cuando el material puede ser muy limitado para ello, los resultados pueden tener algunas consecuencias clínicas que han hecho que el autor modifique con carácter provisorio algunos de sus rígidos principios del tratamiento. La modificación más importante corresponde a la terapia en los casos de necrosis pulpar en dientes con ápice incompletamente desarrollado.

En lugar de tratar de obtener completamente la totalidad del conducto después del desbridamiento y la esterilización, se emplea el método descripto en este trabajo. Los resultados clínicos han sido estimulantes en el sentido que parece haber tenido lugar un posterior desarrollo del ápice según lo prueban las radiografías de control.

Con respecto al agente EDTAC, las observaciones no mostraron que su aplicación en el conducto y en el periodonto apical causara ningún daño irreversible a los tejidos o interfirieran los procesos de cicatrización en grado apreciable.

REFERENCES

Ackerman, L. V., 1959: The surgical pathology. 2. ed. St. Louis.

Allgöwer, M., 1949: Verwendungsmöglichkeiten von Blut und Blutbestandteilen in der Wundbehandlung. Helv. chir. Acta *16:* 249—225.

—»— 1956: The cellular basis of wound repair. Springfield, Ill.

Aschoff, L., 1892: Ueber den Aufbau der menschlichen Thromben und das Vorkommen von Plättchen in den blutbildenden Organen. Arch. path. Anat. *130:* 93—145.

Biolcati, E. L., R. Erausquin y O. A. Maisto, 1942: Control del tratamiento de conductos radiculares. An. Ateneo Inst. M. Odont. 1942/43/44.

Carrel, A., 1930: The process of wound healing. Proc. Inst. Med. Chicago *8:* 62.

Coolidge, E. D. and R. G. Kesel, 1956: A textbook of endodontology including the clinical pathology and treatment of the dental pulp pulpless teeth. London.

Dible, J. H., 1958: Organisation and canalisation in arterial thrombosis. J. Path. Bact. *75:* 1—7.

Edwards, R. H., S. S. Sarmenta and G. M. Hass, 1960: Stimulation of granulation tissue growth by tissue extracts. Study in intramuscular wounds in rabbits. Arch. Path. *69:* 286—302.

Engel, H., 1950: Die Behandlung infizierter Wurzelkanäle und Granulome nach der Methode von Walkhoff. Vergleichend röntgenologisch-histologische Untersuchungen. Schweiz. Mschr. Zahnheilk. *60:* 1077—1109.

Follis, R. H., 1951: Histochemical studies on cartilage and bone. II. Ascorbic acid deficiency. Bull. Johns Hopkins Hosp. *89*:9—20.

Fränkel, R., 1929: Hauptwirkungen der allgemeinen Regenerationshormone. Arch. klin. Chir. *155:* 215—231.

— » — 1931: Über die „Regel des anderen Weges" bei der Wirkung von Abbaustoffen auf die Regeneration. Arch. klin. Chir. *168:* 524—536.

— » — 1932: Die ursächlichen und die regulierenden Wirkungen der Abbaustoffe auf die Regeneration. Arch. klin. Chir. *169:* 174—493.

Godal, H. C., 1960: The effect of EDTA on human fibrinogen and its significance for the coagulation of fibrinogen with thrombin. Scan. J. clin. Lab. Invest. *12*, suppl. 53.

Goldner, J., 1938: A modification of the Masson trichrome technique. Amer. J. Path. *14:* 247.

Gould, B. S., 1960: Ascorbic acid and collagen fiber formation. Vitam. and Horm. *18:* 89—120.

Gould, B. S., G. Manner, H. M. Goldman and J. M. Stolman, 1960: Some aspects of collagen formation. Ann. N. Y. Acad. Sci. *85:* 385—398.

Honegger, I., 1932: Histologische Untersuchungen über die apikalen Heilungsvorgänge entpulpter Zähne nach Einwirkung von Chlorphenol-Kampfermenthollösung und Chlorphenol-Kampfermenthol-Jodoformpasta (Methode *Walkhoff*) auf das gesunde Periodontium. Schweiz. Mschr. Zahnheilk. *42:* 761—807.

Hyakusoku, H., 1959: Effect of filling material on healing of periapical tissues in treatment of human infected root canal. Tokyo dent. Coll. Bull. oral Path. *4:* 51—78.

Kovac, W. und B. Rudas, 1960: Experimentell-histologische Untersuchungen über die Dynamik des Granulationsgewebes. Virchows Arch. path. Anat. *333:* 81—96.

Kukidome, K., 1957: Histopathological study on healing of periapical tissues after infected root canal treatment of human teeth. Tokyo dent. Coll. Bull. oral Path. *2:* 65—87.

Kuroiwa, K., 1960: Experimental study on root canal filling using new filling material, N_2 and AN_2. Tokyo dent. Coll. Bull. oral Path. *4:* 45—73.

Langeland, K., 1957: Tissue changes in the dental pulp. An experimental histologic study. Odont. T. *65:* 239—386.

Lorin-Epstein, M. J., 1927: Über einige allgemeine Faktoren der Wiederherstellungsprozesse und ihre Bedeutung für die chirurgische Pathologie. Arch. klin. Chir. *144:* 632—666.

Löe, H. and J. Waerhaug, 1961: Experimental replantation of teeth in dogs and monkeys. Arch. oral. Biol. *3:* 176—184.

Matsumiya, S. and M. Kitamura, 1960: Histo-pathological and histo-bacteriological studies of the relation between the condition of sterilization of the interior of the root canal and the healing process of periapical tissues in experimentally infected root canal treatment. Bull. Tokyo dent. Coll. *1:* 1—19.

Nygaard Östby, B., 1939: Über die Gewebsveränderungen im apikalen Paradentium des Menschen nach verschiedenartigen Eingriffen in den Wurzelkanälen. (Eine klinische, röntgenologische und histo-pathologische Studie.) Det Norske Videnskaps-Akademi i Oslo, 1939, No. 4.

—»— 1944: Om vevsforandringer i det apikale paradentium hos mennesket ved rotbehandling. (Nye klinisk-röntgenologiske og histo-patologiske studier.) Tissue changes incident to root canal treatment. Det Norske Videnskaps-Akademi i Oslo, 1944, No. 2.

—»— 1957: Chelation in root canal therapy. Ethylenediamine tetra-acetic acid for cleansing and widening of root canals. Odont. T. *65:* 3—11.

—»— 1958: A manual in endodontics. Oslo.

Patterson, Samuel S., 1960: *In vivo* and *in vitro* studies of the effect of the disodium salt of ethylene-diamine tetra-acetate on human dentin and its endodontic implications. Typewritten ms., Bloomington, Indiana. Thesis (M.S.D.) Indiana University School of Dentistry, Indianapolis, Indiana.

Ramström, G., 1961: Dinatriumversenatets och svavelsyrans dentinlösande förmåga. Svensk Tandläk. T. *54:* 339—344.

Saitta, S., 1929: Influenza delle vitamine e delle avitaminosi sulla cicatrizzazione delle ferite studiata col metodo grafico. Nota I: Avitaminosi C. Scritti biologici, *4:* 301.

Stearns, M. L., 1940: Studies on the development of connective tissue in transparent chambers in the rabbit's ear. I. Amer. J. Anat. *66:* 133—176.

Tollardo, G., 1931: Über Heilungsvorgänge im periapikalen Gebiet nach antiseptischer Wurzelbehandlung. Schweiz. Mschr. Zahnheilk. *41:* 441—487.

Torneck, Calvin D., 1961: Reaction of hamster tissue to drugs used in steriliation of the root canal. Oral Surg. *14:* 730—747.

Weinmann, J. F. and H. Sicher, 1955: Bone and bones. Fundamentals of bone biology. London. 2. ed.

Werthemann, A., 1928: Über den Aufbau der Blutgefässwand in entzündlichen Neubildungen, insbesondere in Pleuraschwarten. (Histologische Studie zur Frage mesenchymaler Differenzierungen.) Virchows Arch. path. Anat. *270:* 605—666.

Woessner, J. F. and B. S. Gould, 1957: Collagen biosynthesis. J. biophys. biochem. Cytol. *3:* 685—695.