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Non-microbial etiology: foreign body reaction maintaining post-treatment apical periodontitis

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The polymerase chain reaction (PCR) is an elegant technology for faithfully replicating and amplifying the master molecule of life, but a valid scientific procedure remains essential without which the resulting data have only very limited value. The presence of microbial infection in the complex apical root canal system is the major cause of post-treatment apical periodontitis in well-treated teeth. However, in rare cases, non-microbial etiological factors, located beyond the root canal system (within the inflamed periapical tissues), can maintain the disease in root-filled teeth. These factors include foreign body reaction to exogenous materials or endogenous cholesterol crystals, a cystic condition of the lesion and extraradicular actinomycotic infections. This article addresses *foreign body reaction* at the periapex, as a pathobiological factor that maintains post-treatment apical periodontitis.

Apical periodontitis is essentially a disease of root canal infection (1–3). The rational treatment of the disease, therefore, has been by elimination or a substantial reduction of the infectious agents from the root canal and the exclusion of further pulp–canal infection by root filling. When the root canal treatment is carried out properly, healing of the periapical lesion usually follows with bone regeneration, which is characterized by a gradual reduction of the radiolucency on follow-up radiographs (4–13). Nevertheless, due to several reasons, complete healing or reduction of the apical radiolucency does not occur in all root-filled teeth. In certain cases, apical periodontitis still persists post-treatment, a condition commonly referred to as 'endodontic failures'. It is widely acknowledged that such post-treatment apical periodontitis occurs when root canal treatment has not adequately controlled and eliminated the infection. Problems, mostly of technical nature, that lead to post-treatment apical periodontitis include: inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, debridement and leaking temporary or permanent fillings (14). Even when the highest standards and the most stringent procedures are followed, apical periodontitis may still persist as asymptomatic radiolucencies, because of the complexity of the root canal system formed by a main and several accessory canals, their apical ramifications and anastomoses (15, 16) that cannot be instrumented, cleaned, medicated and filled with existing instruments, materials and techniques. Further, there are extraradicular etiological factors – located beyond the root canals, within the inflamed periapical tissue – that can interfere with post-treatment healing of apical periodontitis.

The etiology of post-treatment apical periodontitis, persisting as asymptomatic radiolucencies in well-treated teeth, has been ill characterized. Early investigations of periapical biopsies (17–21) have been limited by the use of unsuitable specimens, inappropriate methods and criteria of analysis that failed to yield relevant etiological information. Examples of procedural limitations include: light microscopy without correlative electron microscopic analysis, evaluation of random rather than serial sections, paraffin embedding rather than resin embedding of specimens and assignment of overly broad criteria such as 'bacteria and/or debris', which can encompass many potential etiological agents. During the 1990s, a series of carefully conducted investigations, which have taken into account appropriate case selection and methods, have

shown that there are four biological factors that lead to asymptomatic post-treatment apical periodontitis. These are as follows:

(i) intraradicular infection persisting in the apical root canal system (22);
(ii) extraradicular infection, mostly in the form of periapical actinomycosis (23–26);
(iii) foreign body reaction to extruded root canal filling (27), other foreign materials or endogenous cholesterol crystals (28) and
(iv) cystic lesions (28).

It must be emphasized that of all these factors, persistent infection in the complex root canal system is the major cause of post-treatment apical periodontitis in well-treated teeth (22, 29–31).

In a very recent investigation using a molecular genetic technique (32), all the 22 investigated teeth with 'no symptoms' but unresolved post-treatment apical radiolucencies revealed bacterial DNA in intraradicular samples. In this context, the importance of selecting appropriate cases for investigation cannot be overemphasized. As for instance, five of the 22 teeth 'had temporary (coronal) restorations', a factor that would allow bacterial re-infection of the canals by possible coronal microleakage. Apart from the possible re-infection and/or contamination that can occur even in teeth with permanent coronal restorations, the molecular technique does not differentiate between viable and non-viable organisms, but can pick up a minuscule amount of bacterial DNA that is amplified using the polymerase chain reaction (PCR) (33), resulting in an exponential accumulation of several million copies of the original DNA fragments. The data derived from the molecular technique (32) require very careful interpretation in the light of the technique's many advantages and numerous limitations, so as to avoid reaching an overestimating conclusion that all post-treatment apical periodontitis is caused by the presence of intraradicular infection.

Even greater caution is needed to interpret the published data on the role of non-actinomycotic extraradicular infections in apical periodontitis affecting well root-filled teeth. In addition to the possible 'extraneous' sources, contamination of apical tissue samples with microbes from the infected root canal remains a concern. This is because infectious agents live at the apical foramen of teeth affected by the primary (34) and post-treatment apical periodontitis (22, 35). Microbes in that location can be easily dislodged during surgery and the sampling procedures. Tissue samples thus contaminated with *intraradicular* microbes can give positive results for the presence of an *extraradicular* infection. This may explain the renewed reporting of various microbes in the inflamed periapical tissue of asymptomatic post-treatment lesions by culture (36, 37) and molecular techniques (38, 39) in spite of careful aseptic surgical and sampling procedures.

Apart from the problem of possible contamination of the samples with intraradicular microbes and the inability of the technique to distinguish between viable and non-viable organisms, it also does not differentiate between microbes in phagocytic cells from extracellular microorganisms in periapical tissues. In summary, the problem of how to sample the inflamed periapical tissue and keep it separate from what is on and in the root apex is complex. While molecular genetic techniques offer precision and sophistication, they do not solve the primary problem of how to sample accurately the periapical granuloma without contamination.

In rare cases, independent of a low-grade presence or a total absence of intraradicular microbes, exogenous materials trapped in the periapical area (27, 40), endogenous cholesterol crystals deposited in periapical tissues and a cystic lesion can perpetuate apical periodontitis after root canal treatment. The purpose of this article is to provide a comprehensive review on a *foreign body reaction* at the periapex as a pathobiological factor that can maintain post-treatment apical periodontitis.

Exogenous materials causing foreign body reaction at the periapex

Root-filling materials, other endodontic materials (27, 40) and food particles (41) may reach the periapical tissues and cause a foreign body reaction that may be associated with radiolucency remaining asymptomatic for many years (27).

Gutta percha

The most widely used solid root canal filling material is commercially prepared from gutta percha (*trans*-polyisoprene), the coagulated exudate from *Plaquium gutta* tree of Asia or from similar latex derived from the *Mimisops globsa* tree of South America (42). Dental gutta percha cones are composed of about 20% of gutta percha, 60–75% zinc oxide and varying amounts of

metal sulfates for radioopacity, waxes and coloring agents. Based on *in vivo* implantation experiments in animals, gutta percha cones are considered to be biocompatible and well tolerated by human tissues (43–45). However, this view has not been consistent with the clinical observation that the presence of gutta percha in excess is associated with interrupted or delayed healing of the periapex (4, 6, 9, 11, 27). In general, bulk forms of sterile materials with smooth surfaces placed within bone or soft tissue evoke a fibrous tissue encapsulation, while particulate materials induce a foreign body and chronic inflammatory reaction (46–50). Apart from the particle size, the chemical composition of gutta percha is also of significance. Leaching zinc oxide from gutta percha cones has been shown to be cytotoxic *in vitro* (51, 52), tissue irritating *in vivo* and associated with adjacent inflammatory reaction (53, 54). Tissue response to gutta percha was specifically studied (54) using subcutaneously implanted Teflon cages in which the gutta percha evoked two distinct types of tissue reaction. Large pieces of gutta percha were well encapsulated by collagen and the surrounding tissue was free of inflammation (Fig. 1). In contrast, fine particles of gutta percha evoked an intense, localized tissue response (Fig. 2), characterized by the presence of macrophages and giant cells. The accumulation of macrophages in conjunction with the fine particles of gutta percha is significant for the clinically observed impairment in the healing of apical periodontitis, when teeth are root filled with excess of gutta percha. Pieces of gutta percha cones in periapical tissue can gradually fragment into fine particles that in turn can induce a typical foreign body reaction (27, 54, 55) and activate macrophages (56). The latter are known to release a battery of intercellular mediators that include proinflammatory cytokines and modulators that are involved in bone resorption (57–60).

Further, commercial gutta percha cones may become contaminated with tissue-irritating substances that can initiate a foreign body reaction at the periapex. In a follow-up study of nine asymptomatic persistent apical periodontitis lesions that were removed as surgical block biopsies and analyzed by correlative light and transmission electron microscopy, one biopsy (Fig. 3) revealed the involvement of contaminated gutta percha (27). The radiographic lesion persisted asymptomatically and grew in size during a decade of post-treatment follow-up. The lesion was characterized by the presence of vast numbers of multinucleate giant cells with birefringent inclusion bodies (Fig. 4). In transmission

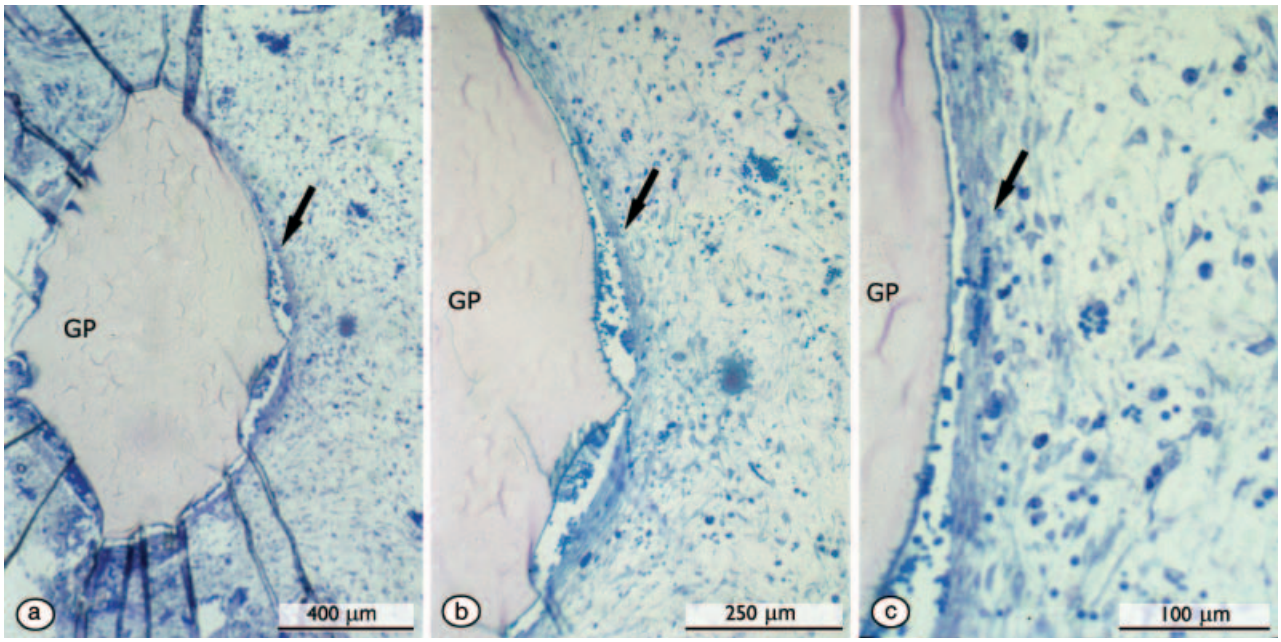


Fig. 1. Guinea-pig tissue reaction to gutta percha (GP) by 1 month after subcutaneous implantation (a). Large pieces of gutta percha are well encapsulated by collagen fibers that run parallel to the surface of the gutta percha particle. The interface of the gutta percha particle and the host tissue (arrow) is magnified in stages in (b, c). The gap between the implant and the collagen capsule is artifactual. Note the non-inflamed, healthy soft delicate connective tissue. Original magnifications: (a) ×42; (b) ×80; (c) ×200.

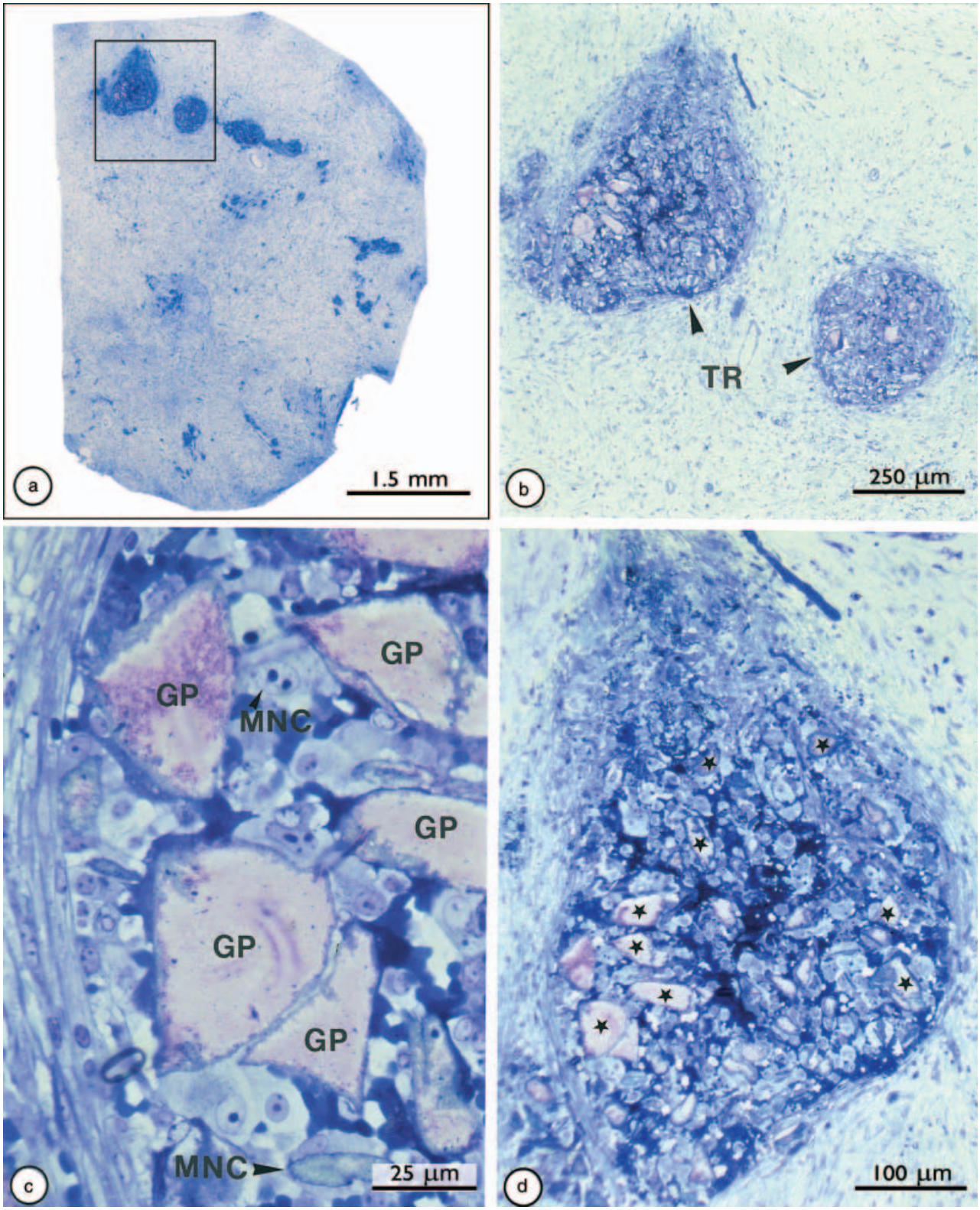


Fig. 2. Disintegrated gutta percha particles that maintain post-treatment apical periodontitis. As clusters of fine particles (a, b) they induce intense circumscribed tissue reaction (TR) around. Note that the fine particles of gutta percha (GP in c, ★ in d) are surrounded by numerous mononuclear cells (MNC). Original magnifications: (a) ×20; (b) ×80; (c) ×750; (d) ×200. From: Nair PNR. Pathobiology of the periapex. In: Cohen S, Burns RC. eds. *Pathways of the Pulp*, 8 edn. St Louis: Mosby, 2002. Reproduced with permission.

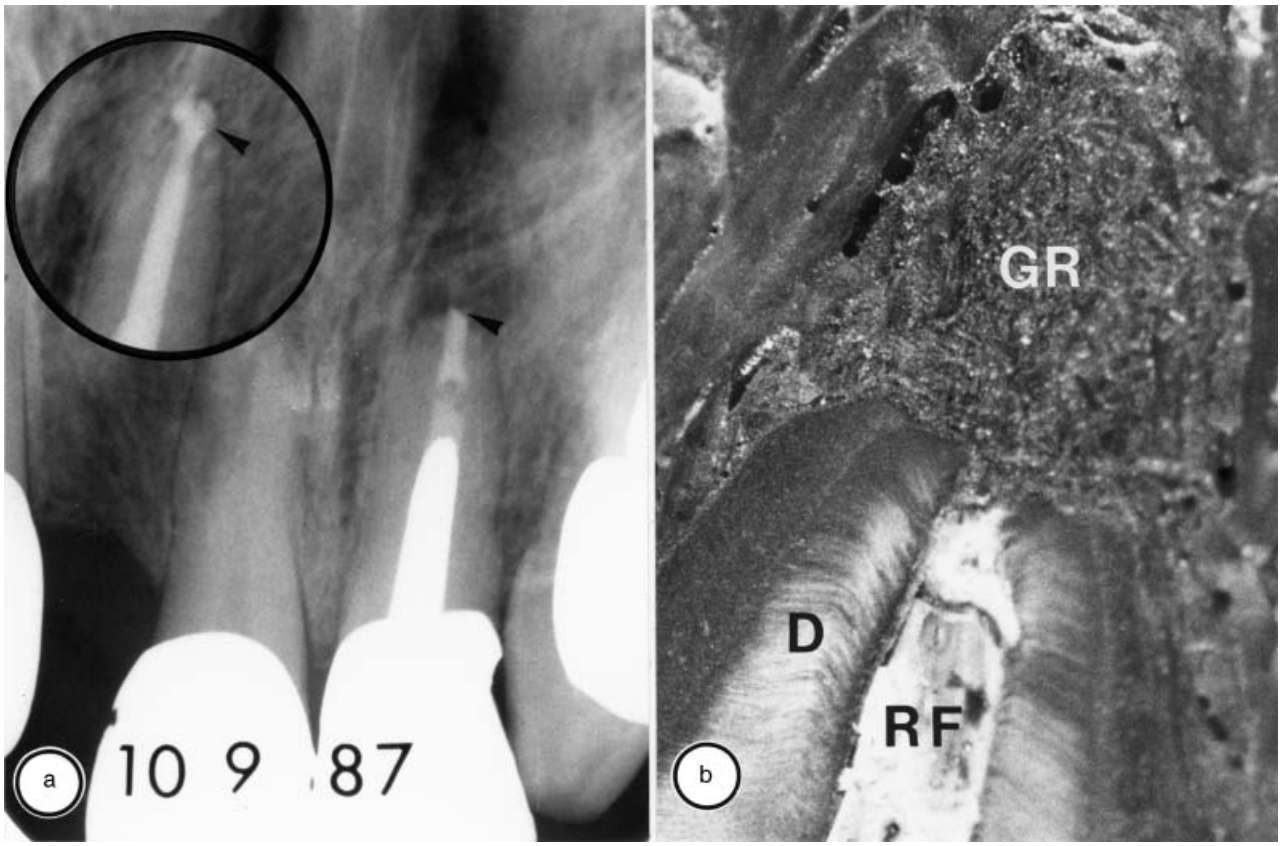
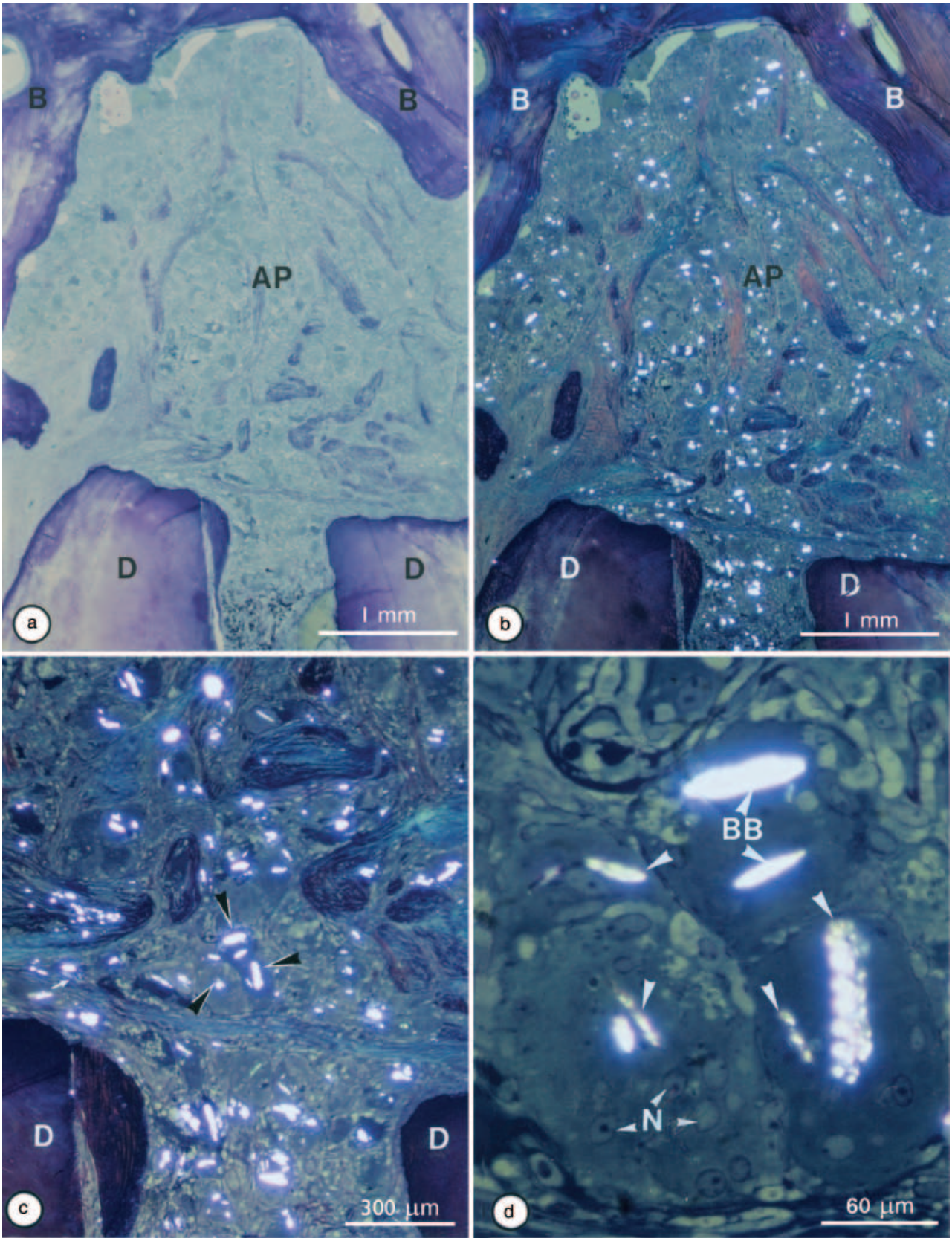


Fig. 3. Two longitudinal radiographs (inset and a) of a root-filled and periapically affected left central maxillary incisor of a 54-year-old man. The first radiograph (inset) taken immediately after root filling in 1977 shows a small excess filling that protrudes into the periapex (arrowhead in inset). Note the excess filling has disappeared in the radiograph taken 10 years later (arrowhead in a) and shortly before surgery was performed. The apical block-biopsy removed by surgery does not show any excess filling as is evident from the macrophotograph of the decalcified and axially subdivided piece of the biopsy (b). RF, root filling; D, dentine; GR, granuloma. Original magnification (b) ×10. From (27). Reproduced with permission.

electron microscopy, the birefringent bodies were found to be highly electron dense (Fig. 5). Energy-dispersive X-ray microanalysis of the inclusion bodies using scanning transmission electron microscopy (STEM) revealed the presence of magnesium and silicon (Fig. 6). These elements are presumably the remnants of talc-contaminated gutta percha that protruded into the periapex and had been resorbed during the follow-up period.

Oral pulse granuloma

Oral pulse granuloma is a distinct histopathological entity (61). It denotes a foreign body reaction to particles of vegetable foods, particularly leguminous seeds such as peas, beans and lentils (pulses) that get lodged in the oral tissues. The lesions are also referred to as the giant cell hyalin angiopathy (61, 62), vegetable granuloma (63) and food-induced granuloma (64). Pulse granuloma has been reported in lungs (65), stomach walls and peritoneal cavities (66). Experimental lesions have been induced in animals by intratracheal, intraperitonial and submucous introduction of leguminous seeds (67, 68). Periapical pulse granulomas are associated with teeth grossly damaged by caries and with a history of endodontic therapy (41, 69). Pulse granuloma is characterized by the presence of intensely iodine and periodic acid-Schiff positive hyaline rings/bodies surrounded by giant cells and inflammatory cells (41, 68–70). The cellulose in plants has been suggested to be the granuloma-inducing agent (67). However, leguminous seeds are the most frequently involved vegetable in such granulomatous lesions. This indicates that other components in pulses, such as antigenic proteins and mitogenic phytohemagglutinins, may also be involved in the pathological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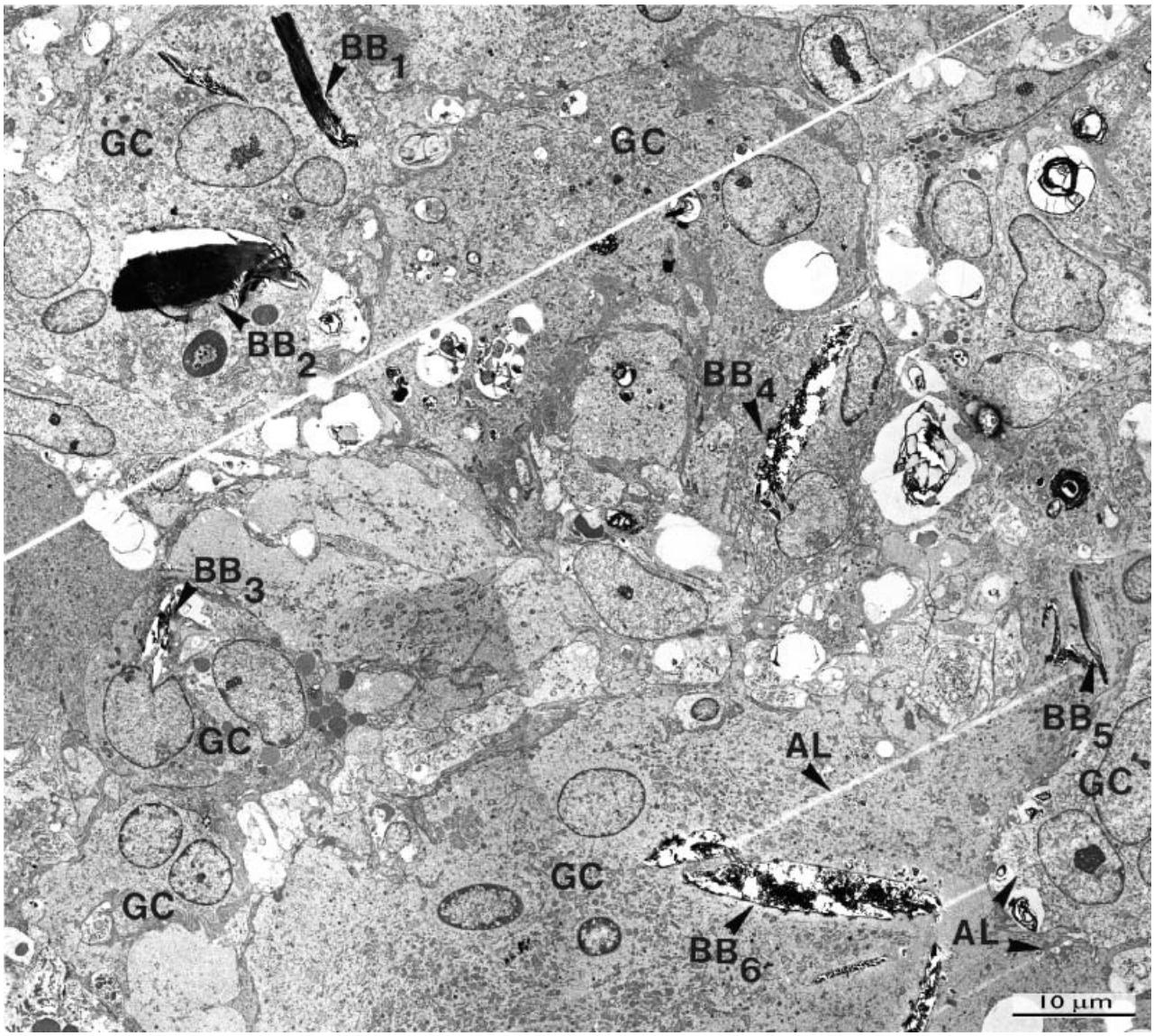


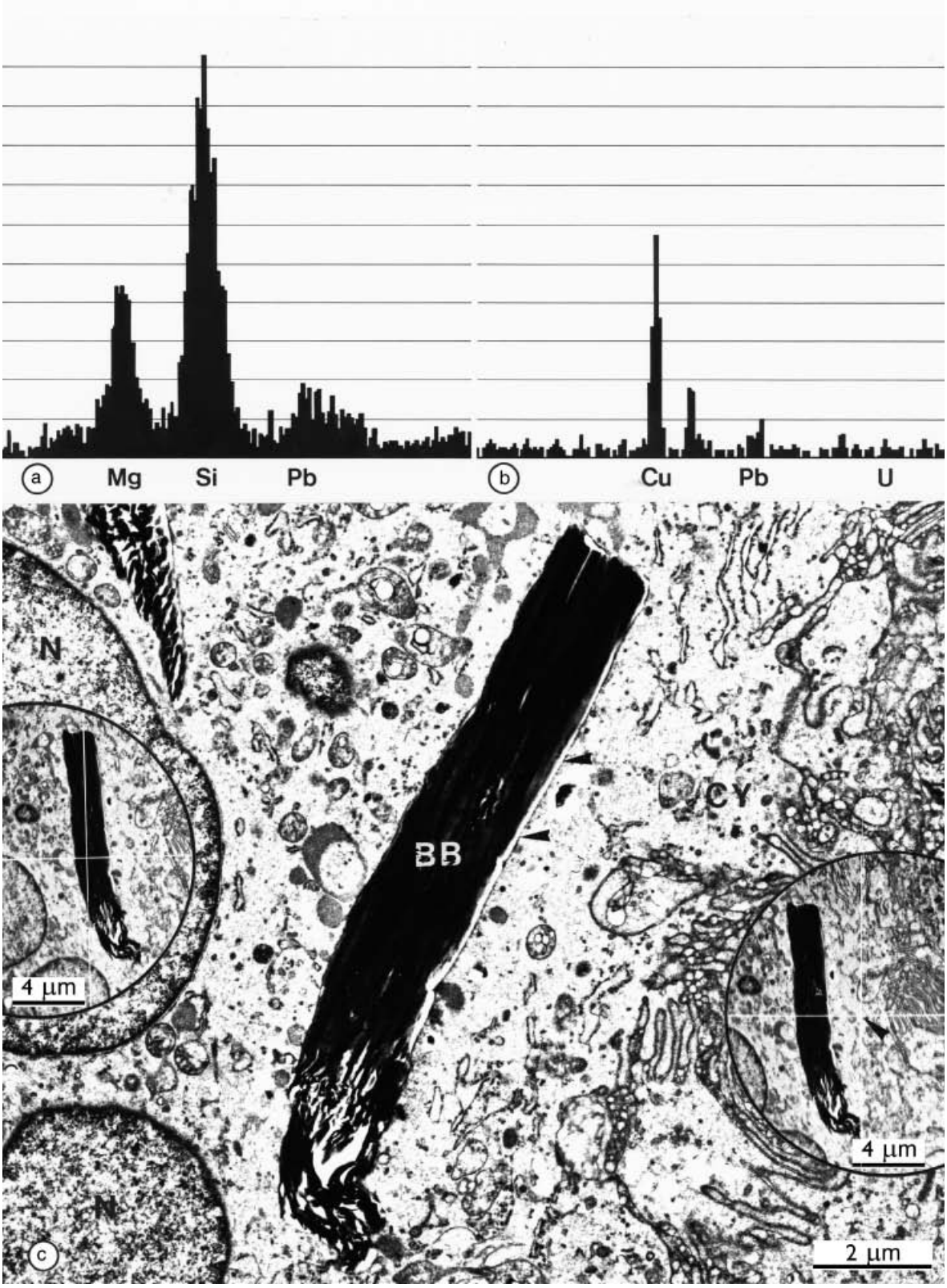
Fig. 5. Low-magnification transmission electron micrograph showing the profiles of several giant cells within the apical periodontitis shown in Figs 3 and 4. Note the presence of many slit-like inclusion bodies (BB_1–BB_6), which contain a highly electron-dense material. This material may remain intact within the inclusion body or may be pushed away from its original site (BB_2) or may appear disintegrated (BB_3 and BB_4) by the tissue processing. Note the lines of artifacts AL, which are created by portions of the electron dense material having been carried away by the knife-edge, leaving tracts behind. Original magnification × 1880. From: (27). Reproduced with permission.

tissue response (67). The pulse granulomas are clinically relevant because particles of vegetable foods can reach the periapical tissues via root canals of teeth exposed to the oral cavity by trauma, caries or endodontic procedures (41). However, the epidemiological incidence of pulse-induced post-treatment apical periodontitis is unknown, as only two such cases have been reported in the literature (41, 70).

Fig. 4. A bright field photomicrograph of a plastic embedded semithin (2 µm thick) section of the apical area shown in Fig. 1b. Note the large apical periodontitis lesion (AP) (a). The same field when viewed in polarized lights (b). Note the birefringent bodies distributed throughout the lesion (b). The apical foramen is magnified in (c) and the dark arrow-headed cells in (c) are further enlarged in (d). Note the birefringence (BB) emerging from slit-like inclusion bodies in multinucleated (N) giant cells. B, bone; D, dentin. Original magnifications: (a, b) × 23; (c) × 66; (d) × 330. From: Nair PNR. Pathology of apical periodontitis. In: Ørstavik D, PittFord TR. eds. *Essential Endodontology.* Oxford: Blackwell, 1998.

Cellulose granuloma

Cellulose granuloma is the term used specifically for pathological tissue reaction to particles of predominantly cellulose-containing materials that are used in endodontic practice (71–74). Endodontic *paper points* are utilized for microbial sampling and drying of root canals. Medicated *cotton wool* has been used in root



canals as well. Particles of these thermo-sterilized materials can easily dislodge or get pushed into the periapical tissue (74) so as to induce a foreign body reaction at the periapex. Therefore, extreme caution should be exercised during clinical manipulation of endodontic paper points (72). The presence of cellulose fibers in periapical biopsies with a history of previous endodontic treatment has been reported (71–73). The overall incidence of cellulose-induced primary or post-treatment apical periodontitis is unknown. This may be partly due to the inconspicuous nature of cellulose material in periapical biopsies and the difficulty in identifying them without the application of special stains or micro techniques. In two histopathological investigations in which 13 biopsies of post-treatment apical periodontitis were examined, all displayed material consistent with cellulose fibers (71, 72). The endodontic paper points and cotton wool consist of cellulose, which is neither digested by humans nor degraded by the body cells. They remain in tissues for long periods of time (73) and evoke a foreign body reaction around them. The particles, when viewed in polarized light, reveal birefringence due to the regular structural arrangement of the molecules within cellulose (71). Paper points infected with intraradicular microorganisms can project through the apical foramen into the periapical tissue (Fig. 7) and allow a biofilm to grow around the paper point (Fig. 7c, d). This will sustain and intensify post-treatment apical periodontitis.

Other foreign materials

Amalgam, endodontic sealer cements and calcium salts derived from periapically extruded calcium hydroxide {$Ca(OH)_2$} also occur in periapical tissues. In a histological and X-ray microanalytical investigation of 29 apical biopsies, 31% of the specimens were found to contain materials compatible with amalgam and endodontic sealer components (40). However, an etiological significance of these materials has not been conclusively shown by experiments. It is possible that these materials might have been co-existing with unidentified etiological agents such as the presence of intraradicular infection in those cases.

Endogenous substances and foreign body reaction

Tissue-irritating endogenous substances are mainly of crystalline fine particular nature. Both endogenous and exogenous crystals induce a pathological tissue response by triggering the cytokine-network-mediated inflammation, hard-tissue resorption and soft-tissue damage. Endogenous crystalline substances that have been shown to cause pathogenic tissue reaction include monosodium urate (gout), calcium phosphate dihydrate (pseudogout), basic calcium phosphate (hydroxylapatite) and cholesterol. Although the presence of cholesterol crystals in apical periodontitis has long been observed to be a common histopathological feature, its etiological significance to post-treatment apical periodontitis has not yet been fully appreciated.

Biology of cholesterol

Cholesterol (75) is a lipid of the steroid family that is present in all animal tissues. The name is derived from *Chole-stereos* meaning 'bile-solid' because of its occurrence in gall stones. Cholesterol was the first steroid to have its structure elucidated. It has the characteristic core of the 'cyclopentanoperhydrophenanthrene' ring (Fig. 8). Cholesterol is an important component of animal cell membranes and is a determinant of membrane properties. It is abundant in 'membrane-rich' tissues (myelin) and cells (secretory cells) and is the precursor of bile acids, provitamin D3 and several hormones (76).

Cholesterol in health

Cholesterol is essential to life and most of the body cholesterol is produced in the liver. The entire body requirement of cholesterol can be met by endogenous

Fig. 6. High-magnification transmission electron micrograph (c) of the intact birefringent body labeled BB_1 in Fig. 5. Note the distinct delimiting membrane around the birefringent body (BB). Energy-dispersive X-ray microanalysis of the electron dense material carried out in scanning transmission electron microscope (STEM: carried out at the point where the two hairlines perpendicular to each other cross in the left inset) revealed the presence of silicon (Si), magnesium (Mg) and lead (Pb) in (a), whereas another site in the neighboring cytoplasm of the same giant cell (arrowhead in the right inset) does not show the presence of Si and Mg (b). Lead and uranium (U) are used for section contrasting, and emission in copper (Cu) is from the section-supporting grid made of copper. Original magnification × 11 000; insets × 3300. From (27). Reproduced with permission.

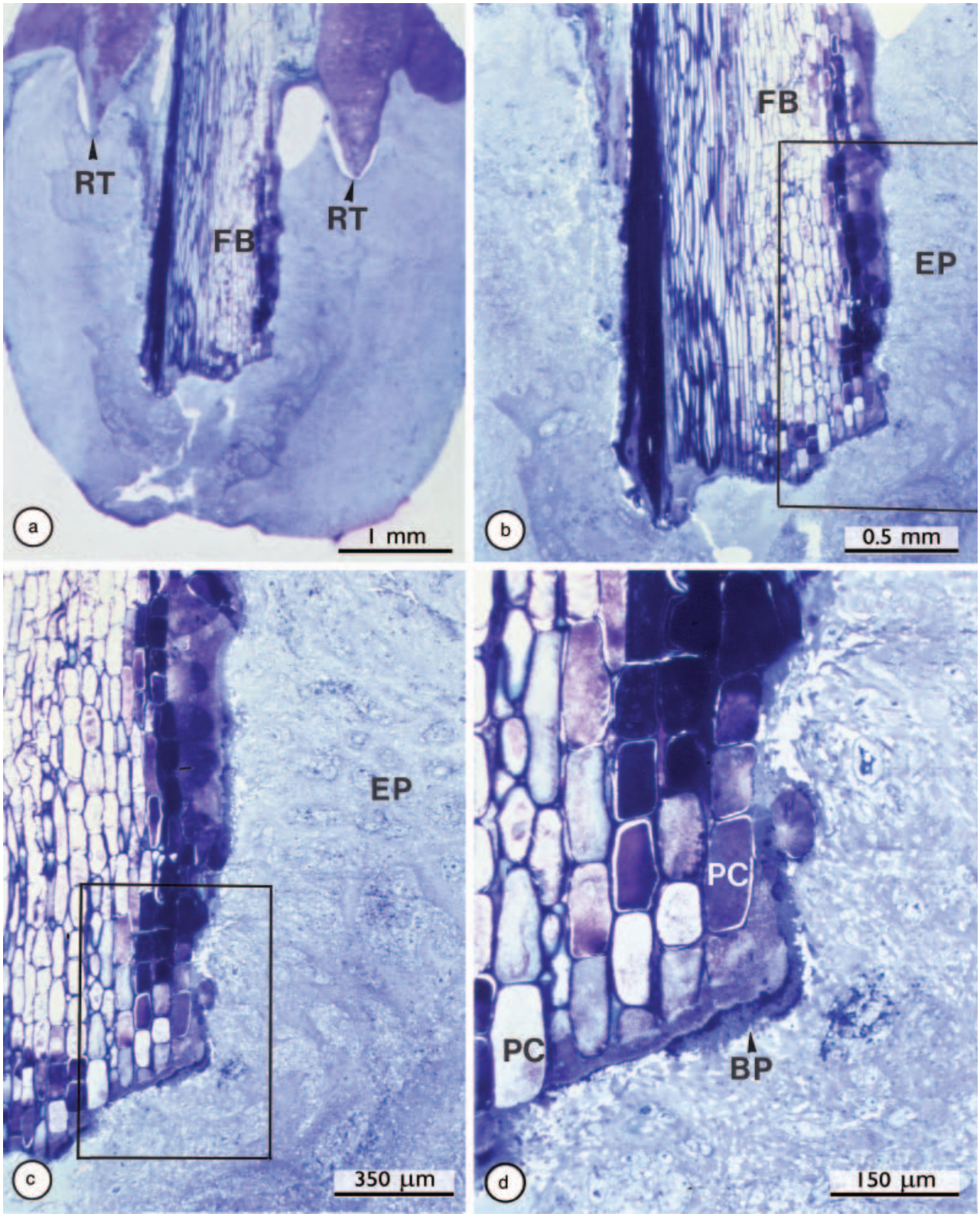


Fig. 7. A massive paper-point granuloma affecting a root-canal-treated human tooth (a). The demarcated area in (b) is magnified in (c) and that in the same is further magnified in (d). Note the tip of the paper point (FB) projecting into the apical periodontitis lesion and the bacterial plaque (BP) adhering to the surface of the paper point. RT, root tip; EP, epithelium; PC, plant cell. Original magnifications: (a) ×20; (b) ×40; (c) ×60; (d)×150. From: Nair PNR. Pathobiology of the periapex. In: Cohen S, Burns RC. eds. *Pathways of the Pulp*, 8th edn. St Louis: Mosby, 2002. Reproduced with permission.

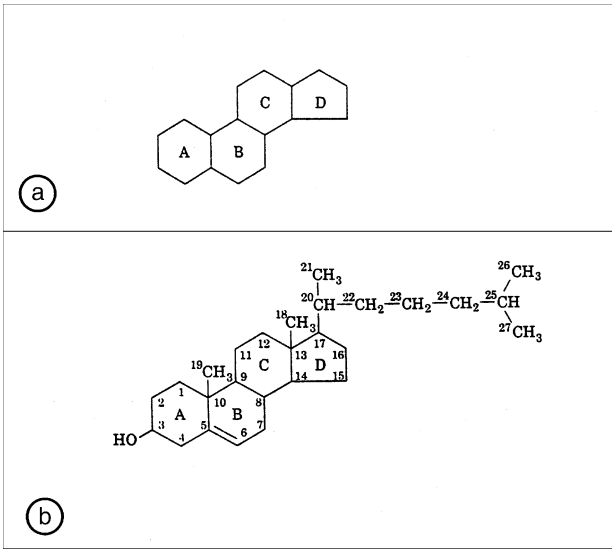


Fig. 8. The parent compound of all steroids is *Cyclopentanoperhydrophenanthrine* with four saturated rings that are designated alphabetically as shown (a). The structural formula of cholesterol (b). Note the four cyclohexane rings and the standard numbering system of all the carbon atoms.

production. Nevertheless, dietary cholesterol is absorbed from the intestine and metabolized. Cholesterol, like other lipids, is insoluble in aqueous solution. Hence, it is transported by the circulation as conjugates of lipoproteins. The latter are globular particles consisting of a core of triglycerides and cholesterol esters that are surrounded by a coating of proteins, phospholipids and cholesterol. On the basis of their functional and physical properties, lipoproteins have been classified into four major categories: (i) chylomicrons, transporting dietary (exogenous) cholesterol from the intestine to the tissues; (ii) very low-density lipoprotein (VLDL), carrying endogenous cholesterol from the liver to the tissues; (iii) low-density lipoprotein (LDL), transporting cholesterol from tissues to the liver and (iv) high-density lipoprotein (HDL), helping the removal of cholesterol from the tissues to the liver.

Dietary cholesterol

Cholesterol and other dietary lipids pass through the mouth and stomach largely untouched by the digestive process. This is in part due to the insolubility of cholesterol in an aqueous medium. In the small intestine, cholesterol is emulsified by entrapment into bile salt micelles, thereby making it accessible to partial digestion by enzymes and eventual absorption by intestinal cells. The absorbed cholesterol and triglycerides are then assembled by the intestinal cells into the largest of the lipoprotein conjugates, the chylomicrons. Via lymph and blood circulation, the chylomicrons are transported to tissues throughout the body. They adhere to the binding sites on the inner surface of the capillary endothelium in skeletal muscle and adipose tissue, where most of the triglyceride component is removed. As a result, the chylomicrons become smaller, greater in density and enriched in cholesterol content. These are chylomicron remnants that dissociate from the capillary endothelium to re-enter the blood circulation. On reaching the liver, much of the exogenous cholesterol in the chylomicron remnants are used by the liver to make bile salts or mixed with cholesterol synthesized by the liver for export to distant organs and tissues.

Endogenous cholesterol

Hepatic cells produce cholesterol from acetic acid. This endogenous cholesterol is mixed with part of the exogenous cholesterol arriving in chylomicron remnants, repacked in VLDL and exported to tissues via the blood. As the VLDL passes through tissues, the cells degrade it by partial consumption of some of its components. Consequently, the VLDL gradually changes. It decreases in size but increases in density to become the intermediate density lipoprotein (IDL), which is a transitional lipoprotein in the pathway. IDL ultimately enters the blood circulation where it is converted to LDL, the major package of blood-borne cholesterol transport from tissues to the liver. It is known to be directly involved in the build-up of plaques on the vascular walls that eventually lead to atherosclerosis. Therefore, LDL is popularly known as 'bad' cholesterol.

LDL is made from IDL by the action of the HDL that is present in blood. Unlike the other types of lipoproteins, HDL is assembled and released into the circulation by extrahepatic tissue cells from components largely obtained by the degradation of other lipoproteins and cell membranes. HDL functions as a cholesterol scavenger and is crucial for the removal of cholesterol from the tissues to the liver. This capability is largely beneficial to the body. Therefore, HDL is known as the 'good' cholesterol.

As pointed out earlier, one of the liver's important products from cholesterol is bile salts, which facilitate absorption of dietary cholesterol and fat from the intestine. Much of the bile salts are salvaged and recycled in the intestine. However, because the reabsorption of bile salts is not 100% efficient, a small amount of bile salts is excreted. In the large intestine, the bile-salt–cholesterol is reduced by bacteria to coprosterol, which is the only form in which cholesterol is excreted from the body, and the liver is the only organ capable of disposing off significant quantities of cholesterol (77).

Cholesterol in disease

Excessive blood level of cholesterol is suspected to play a role in atherosclerosis as a result of its deposition in the vascular walls (76, 78). It is characterized by atheromas (*athere* = mush) that upon sectioning exude a creamy yellow substance rich in cholesterol esters. Atherosclerosis is a chronic, progressive, multifactorial disease that begins as an intracellular deposition of cholesterol in previously damaged sites on the inner arterial walls. The lesions eventually become fibrous calcified plaques. The consequent hardening and narrowing of the arteries promote the formation of intravascular blood clots and infarction of the dependent tissue. Although atheromas can develop in many different blood vessels, they are most common in the coronary arteries. The resultant myocardial infarction is usually fatal and is the most common cause of death in western industrialized nations (77).

Local deposition of crystalline cholesterol also occurs in other tissues and organs, as in the case of otitis media and the 'pearly tumor' of the cranium (79). In the oral region, accumulation of cholesterol crystals occurs in apical periodontitis lesions (28, 80–85) with clinical significance in endodontics and oral surgery (28, 86).

Cholesterol in apical periodontitis

Apical periodontitis lesions often contain deposits of cholesterol crystals appearing as narrow, elongated tissue clefts in histopathological sections. The crystals dissolve in fat solvents used for the tissue processing and leave behind the spaces they occupied as clefts. The reported prevalence of cholesterol clefts in apical periodontitis varies from 18% to 44% (80, 81, 84, 85). The crystals are believed to be formed from cholesterol released by: (i) disintegrating erythrocytes of stagnant blood vessels within the lesion (84), (ii) lymphocytes, plasma cells and macrophages that die in great numbers and disintegrate in chronic periapical lesions (85) and (iii) the circulating plasma lipids (81). All these sources may contribute to the concentration and crystallization of cholesterol in the periapical area. Nevertheless, inflammatory cells that die and disintegrate within the lesion may be the major source of cholesterol, as a result of its release from membranes of such cells in long-standing lesions (28, 87). The crystals are initially formed in the inflamed periapical connective tissue, where they act as foreign bodies and provoke a giant cell reaction.

In histological sections, numerous multinucleated giant cells can be observed around the cholesterol clefts (Fig. 9). When a large number of crystals accumulate in the inflamed connective tissue they passively move in the direction of least resistance. If the lesion happens to be a radicular cyst, the crystals move in the direction of the epithelium-lined cyst cavity, as the outer collagenous capsule of the lesion is much tougher for the crystals to move through. The slow 'glacier-like' movement of the crystal-mass erodes the epithelial lining and empties the crystals into the cyst lumen (Fig. 9).

Radicular cysts (88) and apical granulomas (82) in which cholesterol clefts form a major component are referred to as 'cholesteatoma'. The term originates from general pathology where it refers to a local accumulation of cholesterol crystals that cause discomfort and dysfunction of the affected organs (79). Therefore, it has been suggested (28) to use it more specifically as 'apical choleastoma' so as to distinguish the condition from cholesteatoma affecting other tissues and organs.

In vivo reaction to cholesterol

There have been several animal studies on the tissue reaction to cholesterol crystals in conjunction with the role of the crystals in cardiovascular diseases. Cholesterol crystals are intensely sclerogenic (89, 90). They have been shown to induce granulomatous lesions in dogs (91), mice (89, 90, 92–94) and rabbits (92, 95, 96). The cholesterol was applied in those studies by direct injection of its suspension into arterial walls (91), by subcutaneous deposition of cholesterol crystals (89, 90, 93, 94) or by subcutaneous implantation of

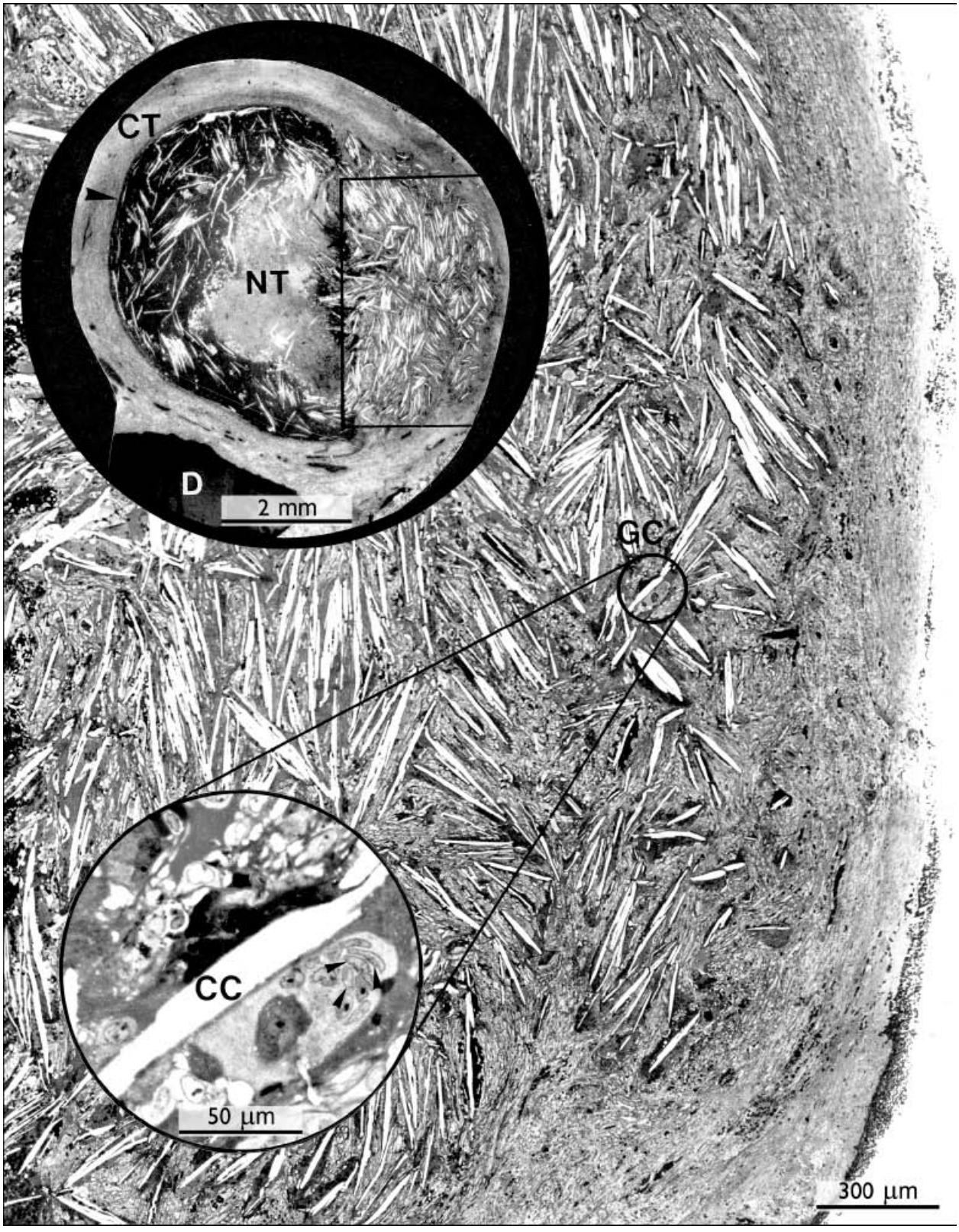


Fig. 9. Cholesterol crystals and cystic condition of apical periodontitis as potential causes for endodontic failures. Overview of a histological section (upper inset) of an asymptomatic apical periodontitis that persisted after conventional root canal treatment. Note the vast number of cholesterol clefts (CC) surrounded by giant cells (GC) of which a selected one with several nuclei (arrowheads) is magnified in the lower inset. D, dentine; CT, connective tissue; NT, necrotic tissue. Original magnifications: ×68; upper inset ×11; lower inset × 412. From: Nair PNR. *Aust Endod J* 1998: 25: 19–26. Reproduced with permission.

absorbable gelatin sponge that had been saturated with cholesterol in ether and the solvent was allowed to evaporate before the implantation (92, 96). These studies consistently showed that the cholesterol crystals were densely surrounded by macrophages and giant cells.

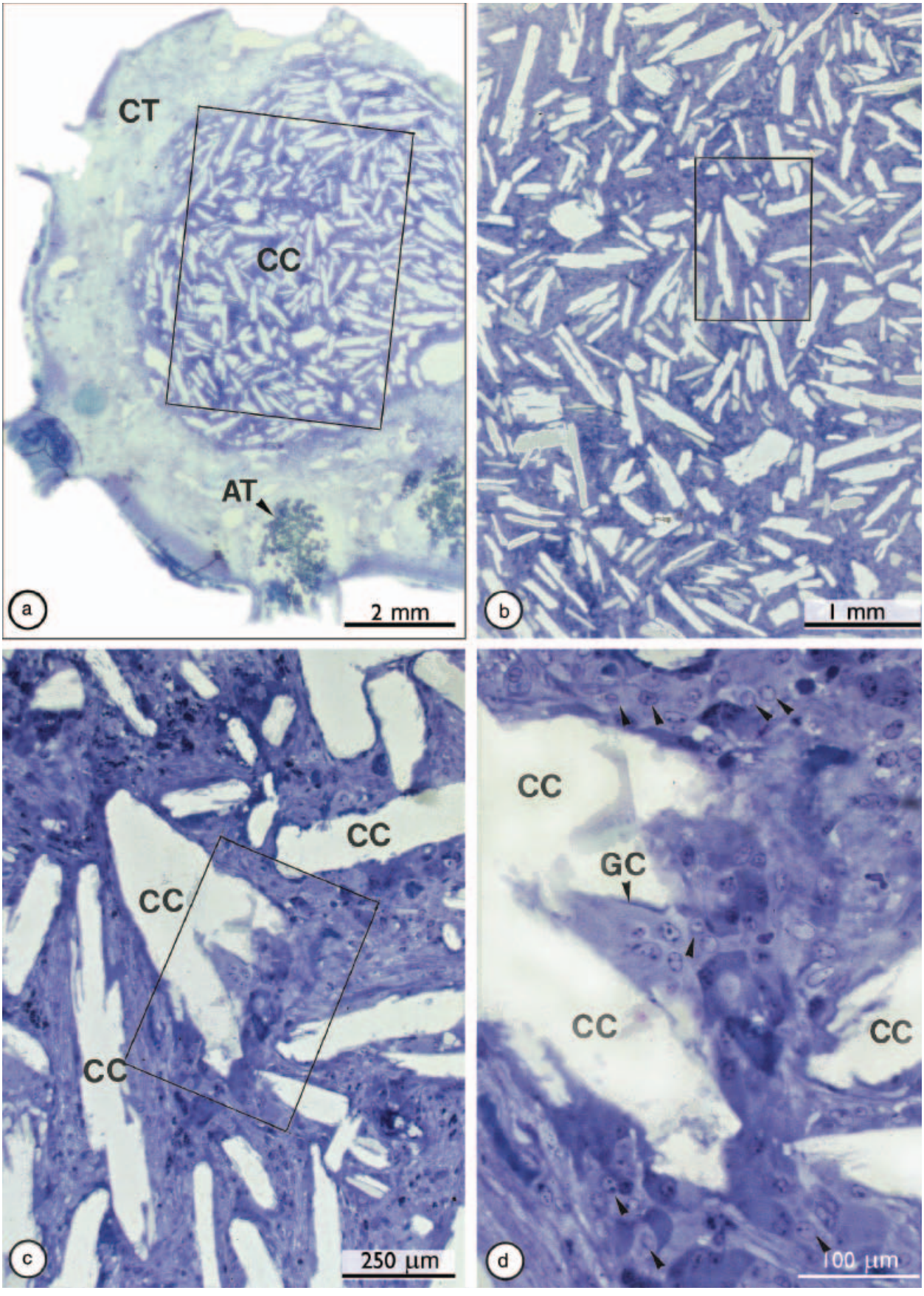
To the author's knowledge, there is only one experimental study reported in the literature that specifically addressed the potential association of cholesterol crystals and non-resolving apical periodontitis lesions (97). In this *in vivo* study in guinea-pigs, the tissue reaction to cholesterol crystals was investigated using a Teflon cage model (98) that facilitated the intact surgical retrieval of the cholesterol crystals with the surrounding host tissue after the experimentation. The study was designed to answer the question as to whether aggregates of cholesterol crystals would induce and sustain a granulomatous tissue reaction in guinea-pigs. Pure cholesterol crystals, prepared to a mushy form, were placed in Teflon cages that were implanted subcutaneously in guinea-pigs. The cage contents were retrieved after 2, 4 and 32 weeks of implantation and processed for light and electron microscopy. The cages revealed delicate soft connective tissue that grew in through perforations on the cage wall. The crystals were densely surrounded by numerous macrophages and multinucleated giant cells (Figs 10 and 11), forming a well-circumscribed area of tissue reaction. The cells, however, were unable to eliminate the crystals during an observation period of 8 months. The tissue response to cholesterol crystals observed in the investigation was totally consistent with the findings of previous morphological investigations (89–93, 96).

The congregation of macrophages and giant cells around cholesterol crystals in the absence of other inflammatory cells, such as neutrophils, lymphocytes and plasma cells suggests that the crystals induced a typical foreign body reaction (27, 54, 55). While most of the macrophages may be freshly recruited blood monocyte population (99, 100), the giant cells are of local origin. Radioactive labeling studies (101, 102) have conclusively shown that giant cells are monocyte derivatives formed by fusion of macrophages. Investigations on the cytogenesis of multinucleate giant cells around cholesterol crystals in subcutaneous implants suggest that they are formed by a process of 'circumfusion' (90) of macrophages around individual crystals. Once formed, the giant cells can also enlarge in size by synchronous division of their nuclei (103).

Body cells cannot eliminate cholesterol crystals

It is of clinical interest to know to what extent the body cells are able to eliminate locally accumulated cholesterol crystals. Such degradation should occur via the phagocytic and/or biochemical pathways. In addition to their central role in immunological defense and inflammation, macrophages are efficient phagocytes (104) capable of ingesting and killing microorganisms, scavenging dead cells and necrotic tissue and removing small foreign particles (105). Cells belonging to the mononuclear phagocytic system (106) are involved in lipid uptake (107). Macrophages have been shown to internalize cholesterol crystals *in vitro* (90, 107). Fine suspensions of cholesterol crystals administered intraperitoneally in rats were found in sternal lymph node macrophages (108, 109). In this apparently phagocytic intake of particulate cholesterol, the sizes of the crystals must have been appropriately small for the macrophages to ingest them. However, when macrophages encounter larger foreign particles (27, 54) or cholesterol crystals (89–93, 96) they form multinucleate giant cells. The presence of giant cells in cholesterol granuloma is a clear sign of the large size of the crystals in relation to macrophages. However, the giant cells are poor phagocytes (102, 110), their phagocytic efficiency declining with increasing size of the cells (111, 112). The degradative power of multinucleate giant cells is mainly vested in their ability to resorb intrinsic and extrinsic substrates. Resorption is a highly specialized cellular activity in which the destruction of suitable substrates occurs extracellularly at the specialized cell/substrate interface by biochemical means.

Fig. 10. Photomicrograph (a) of guinea-pig tissue reaction to aggregates of cholesterol crystals after an observation period of 32 weeks. The rectangular demarcated areas in (a), (b) and (c) are magnified in (b), (c) and (d), respectively. Note the rhomboid clefts left by cholesterol crystals (CC) surrounded by giant cells (GC) and numerous mononuclear cells (arrowheads in d). AT, adipose tissue; CT, connective tissue. Original magnifications: (a) × 10; (b) × 21; (c) × 82; (d) × 220. From: Nair PNR. *Aust Endod J* 1998: 25: 19–26. Reproduced with permission.


CT
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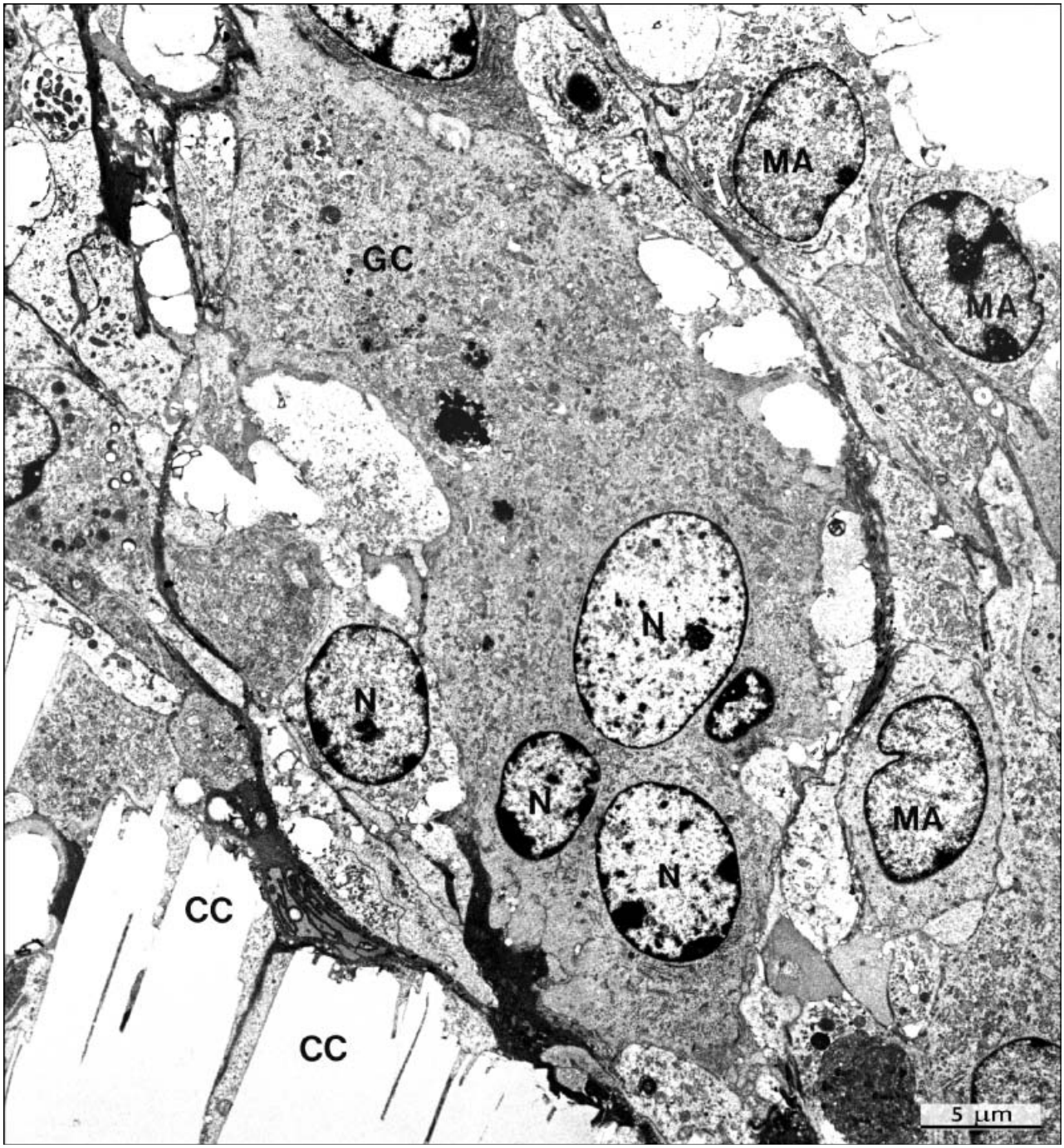

Fig. 11. Ultrastructure of guinea-pig tissue reaction to cholesterol crystals (CC) in cages that were removed 32 weeks after implantation. Note a large multinucleated (N) giant cell (GC) and numerous macrophages (MA) around the crystals. Original magnification × 4600. From: Nair PNR, *Aust Endod J* 1998: 25: 19–26. Reproduced with permission.

In order to degrade tissue deposits of cholesterol crystals, the surrounding cells should have the ability to attack the crystals chemically so as to disperse them into the surrounding tissue fluid or to make them accessible to the cells themselves. Cholesterol crystals are highly hydrophobic and their dispersal would necessitate making them hydrophilic and 'soluble' in an aqueous medium (89). The granulomatous and sclerogenic effects of cholesterol crystals can be prevented by the incorporation of phospholipids into subcutaneous

implants of cholesterol (93). This beneficial effect of phospholipids has been attributed to their 'detergent' property and their role as donors of polyunsaturated fatty acids during esterification of the cholesterol (89, 94). The giant cells and macrophages are known to esterify and mobilize cholesterol in a lipid droplet form (90). Macrophages can convert particulate cholesterol into a soluble form by incorporating it into a lipoprotein vehicle (107, 113), so that the cholesterol can be readily esterified or added into the lipoprotein pool in circulation.

These cell biological findings obviously support the possible ability of macrophages and giant cells to degrade particulate cholesterol. But they are not consistent with the histopathological observation of spontaneous (28, 82, 114) and experimentally induced (89–93, 96) cholesterol granulomas. The characteristic feature of such lesions is the accumulation of macrophages and giant cells around the cholesterol clefts and their persistence for long periods of time. Therefore, it is reasonable to assume that the macrophages and the multinucleate giant cells that congregate around cholesterol crystals are unable to destroy the crystals in a way beneficial to the host (97). It is in this context that one should interpret the clinical significance of massive accumulation of cholesterol crystals in apical periodontitis lesions. The macrophages and giant cells that surround cholesterol crystals are not only unable to degrade the crystalline cholesterol, but are major sources of apical inflammatory and bone resorptive mediators. Bone resorbing activity of cholesterol-exposed macrophages due to enhanced expression of IL-1_{α} has been experimentally shown (115). Based on these considerations, it was concluded in a long-term longitudinal follow-up of a case that 'the presence of vast numbers of cholesterol crystals…would be sufficient to sustain the lesion indefinitely' (28). The animal experimental results and other evidence presented from the literature confirm this assumption.

Clinical relevance and concluding remarks

Because intraradicular infection is the primary and major cause of apical periodontitis, the aim of conventional endodontic treatment is to eliminate infectious agents from the root canal and to prevent re-infection by root filling. However, the tissue dynamics of apical periodontitis persisting from foreign body reaction are not dependent on the presence or absence of infectious agents or other irritants in the root canal. The macrophages and giant cells that accumulate in sites of foreign body reaction are not only unable to degrade the foreign materials and endogenous substances that sustain the reaction but are also major sources of inflammatory and bone resorptive cytokines and other mediators. There is clinical and histological evidence that the presence of tissue-irritating foreign materials at the periapex, such as extruded root-filling materials, endodontic paper points, particles of foods and accumulation of endogenous cholesterol crystals, adversely affect post-treatment healing of the periapical tissues. The overall prevalence of foreign body reaction at the periapex is currently unknown, but the occurrence of such cases may be very rare. Nevertheless, endodontic clinical situations involving foreign bodies can result in 'prolonged…troublesome and disconcerted course of events' (74).

Therefore, it may be concluded that initiation of a foreign body reaction in the periapical tissues by exogenous materials or endogenous cholesterol delays or prevents post-treatment healing. In well-treated teeth with adequate root filling, an orthograde retreatment is unlikely to resolve the problem, as it does not remove the offending objects and substances that exist beyond the root canal (27, 28, 40, 71). Currently, a clinical differential diagnosis for the existence of these extraradicular agents of post-treatment apical periodontitis is not possible. Further, the great majority of post-treatment apical periodontitis cases are caused by infection persisting in the complex apical portion of the root canal system (15, 16). It is not guaranteed that an orthograde retreatment of an otherwise well-treated tooth can eradicate the intraradicular infection. Therefore, a clinician faced with a patient presenting an asymptomatic, persistent, post-treatment periapical radiolucency should consider the necessity of removing extraradicular offending factors by way of apical surgery (116), in order to improve the long-term outcome of treatment. A surgical treatment provides not only an opportunity to remove the extraradicular agents that sustain the apical radiolucency post-treatment but also allows a retrograde approach to any potential infection in the apical portion of the root canal system that can also be eliminated or sealed within the canal by a root-end filling.

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