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The cellular mechanism of ossicular erosion in chronic suppurative otitis media

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Summary

This is the first report of the application of a new examination technique for the assessment of cellular activity during bone resorption in chronic suppurative otitis media (CSOM). A total of nineteen incudes removed during the course of tympanomastoid surgery were studied (retraction pocket: 2; tubo-tympanic CSOM: 4; attico-antral CSOM: 13). The microscopic surface topography of each specimen was examined using the scanning electron microscopy (SEM), and the appearances are interpreted in terms of cortical cellular activity. The results suggest that the mechanism of ossicular erosion in CSOM is similar regardless of the exact type of disease. Extensively pitted areas were seen in all specimens. These pits are morphologically indistinguishable from those characteristic of osteoclastic activity (Howship's lacunae). We conclude that in all causes the surface topography of eroded incudes is consistent with the activity of osteoclasts.

Key words: Ear ossicles; Otitis media; Microscopy, electron, scanning.

Introduction

The precise mechanism of bone erosion in CSOM has been disputed. Various general theories have been proposed, but most controversy has centered on the exact role of the osteoclast in this pathological process. Early investigators readily identified osteoclastic activity in histopathological studies (Grippaudo, 1958; Pollock, 1959). Later studies failed to identify generalized osteoclasis, and this gave support to other theories of resorption. Thomsen *et al.* (1974) found little evidence of osteoclastic activity, and attributed resorption to histiocytic activity within adjacent granulation tissue. Abramson and Huang (1977) argued that bone resorption in CSOM was due to the action of collagenase produced by adjacent inflammatory tissue and/or stratified squamous epithelium. More recent studies have again identified the osteoclast in approximately one half of surgical specimens examined, and in a greater proportion of experimentally produced cholesteatomas (Chole, 1984 and 1988).

The examination techniques used in previous studies have evolved with available technology from conventional light microscopy through to transmission electron microscopy. All, however, involve the examination of serially sectioned specimens which is time consuming and by its nature, restricts the area of diseased bone which can be examined.

SEM has been used previously to examine ear ossicles, but with disappointing results. Brownson and Marovitz (1972) were unable to identify evidence of osteoclastic activity in any ossicular specimen examined using SEM. These and other studies have led to a neglect of SEM in otological research, outside the membranous labyrinth.

In other fields of bone research, particularly where unique specimens cannot be destroyed during examination, investigators persisted with SEM and were able to correlate microscopic surface appearances with the most recent cellular activity on the bone surface (Boyde and Hobdell, 1969; Jones and Boyde, 1977). This has become known as the topographic 'T' principle of SEM (Bromage, 1987). It has been applied successfully in anthropological study (O'Higgins *et al.*, 1991), and also in dental research (Grundy, 1971). The 'T' principle states that the activity of cortical bone during growth and remodelling is distinguishable by its characteristic microscopic surface topography (Bromage, 1982). Under the scanning electron microscope (Fig. 1) areas of osteoclastic erosion are distinguished by the presence of shallow pits with irregular edges. These pits (Howship's lacunae) are commonly found in patches where they often coalesce to form resorptive fields. Surfaces on which the last activity before cell death was deposition show a smoother, fibrous appearance characteristic of mineralized collagen. The degree of mineralization and of mineral organization can be taken to indicate the current activity of depository surfaces. Resting depository surfaces characteristically are well mineralized and organized, whereas active deposition shows less mineral organization and presents a rougher appearance. In contrast to depository surfaces, it is not possible to readily distinguish active from resting resorptive surfaces; both present the pitted appearance characteristic of resorptive fields.

We wish to report on the first application of this technique in otological research, specifically on the cellular mechanism of ossicular resorption in CSOM.

Materials and methods

Nineteen pathological incudes obtained during the

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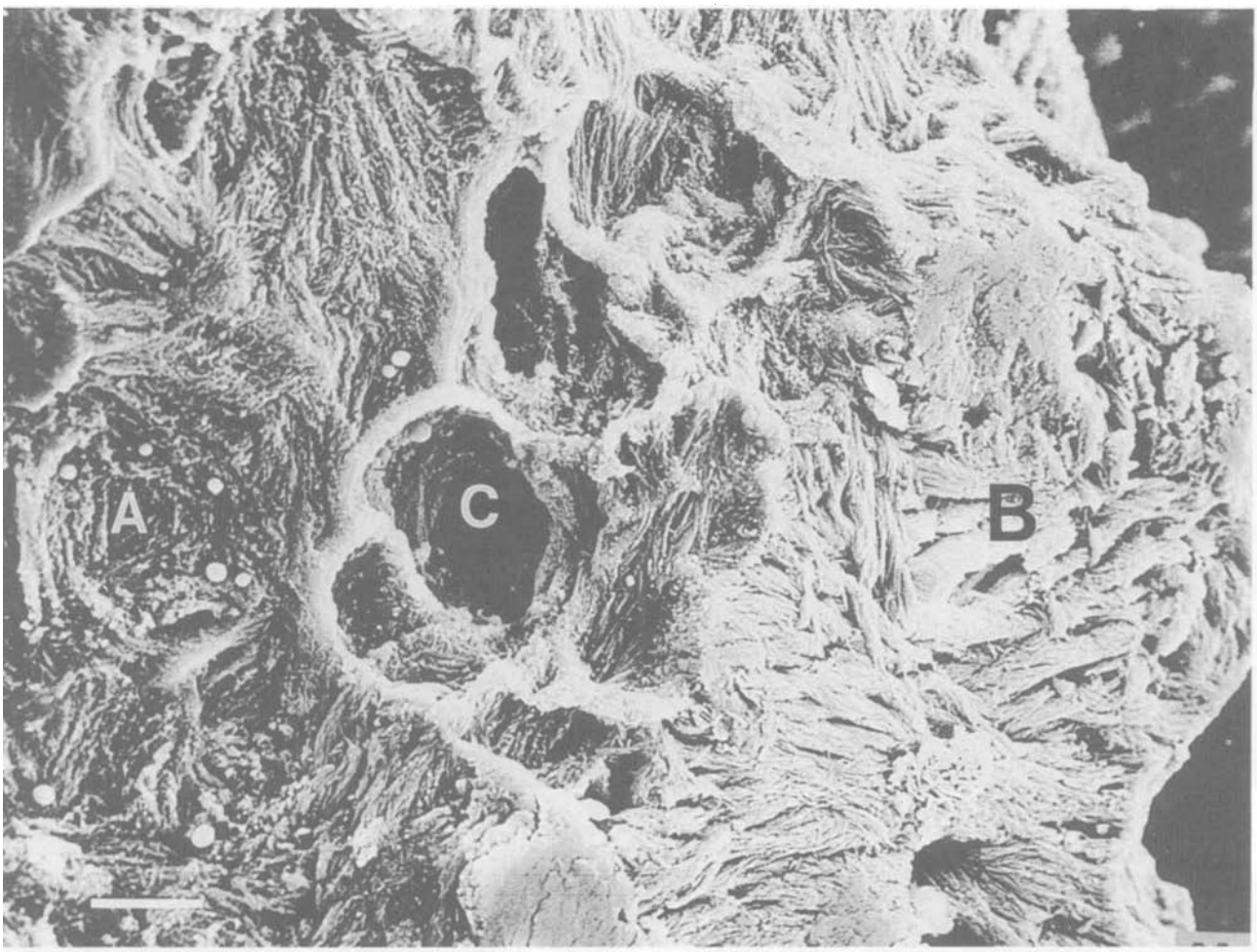

FIG. 1
Characteristic SEM appearance of: A stable bone; B new bone; C osteoclastic pit ('scalloped' appearance). Scale bar = 10 microns.

course of tympanomastoid surgery were examined. The patients from whom the specimens were obtained comprised 6 females and 13 males (age range 5-68 yrs., mean age 33 yrs.). The precise details and nature of disease are summarized in Table I.

Soft tissue is removed from the specimen by immersion in 10 per cent Papain solution at 37°C for 3-5 days. Residual debris is washed off using 5 per cent sodium hypochlorite followed by ethanol. The specimen is then allowed to dry at room temperature, and is 'spatter coated' with gold in an argon vacuum before being mounted on an aluminium base for examination using a JEOL JSM-T20 scanning microscope at magnifications from 35-1500. Patterns of surface activity are mapped according to the 'T' principle of SEM, and representative areas photographed.

TABLE I
SUMMARY OF INCUDES EXAMINED

Retraction pocket	2
Tubo-tympanic CSOM	4
Attico-antral CSOM	13
(6 female, 13 male, mean age 33 yrs. Age range 5-68 yrs)	
Total number examined	19

Results

The appearance of the specimens at low magnification reveals the lenticular and long processes to be the commonest sites of bone erosion (Figs. 2 & 3). In specimens with a greater degree of long process erosion, 'pitting' could also be observed on the body and short process (Fig. 3). When the long process was completely absent, it was replaced by a large erosive pit spreading onto the remaining body and short process (Fig. 3). It was not possible to differentiate disease type on the basis of degree or pattern of erosion.

At higher magnifications the eroded areas all possessed the 'scalloped' appearance characteristic of osteoclasis (Fig. 4). The long process, where present, had the greatest density of osteoclastic pits. With progressive erosion of the long process, a wave of osteoclasis appeared to 'advance' towards the body and short process. This large area of osteoclastic activity coalesced with smaller osteoclastic foci as it enlarged. These small foci were predominantly centred on vascular foramina, and account for the pitting observed on the body and short process at lower magnification (Fig. 3). No erosive pattern except that characteristic of osteoclastic activity could be identified in any specimen and in addition, the mechanism of erosion appeared similar irrespective of the disease type.

There was evidence of limited new bone formation,

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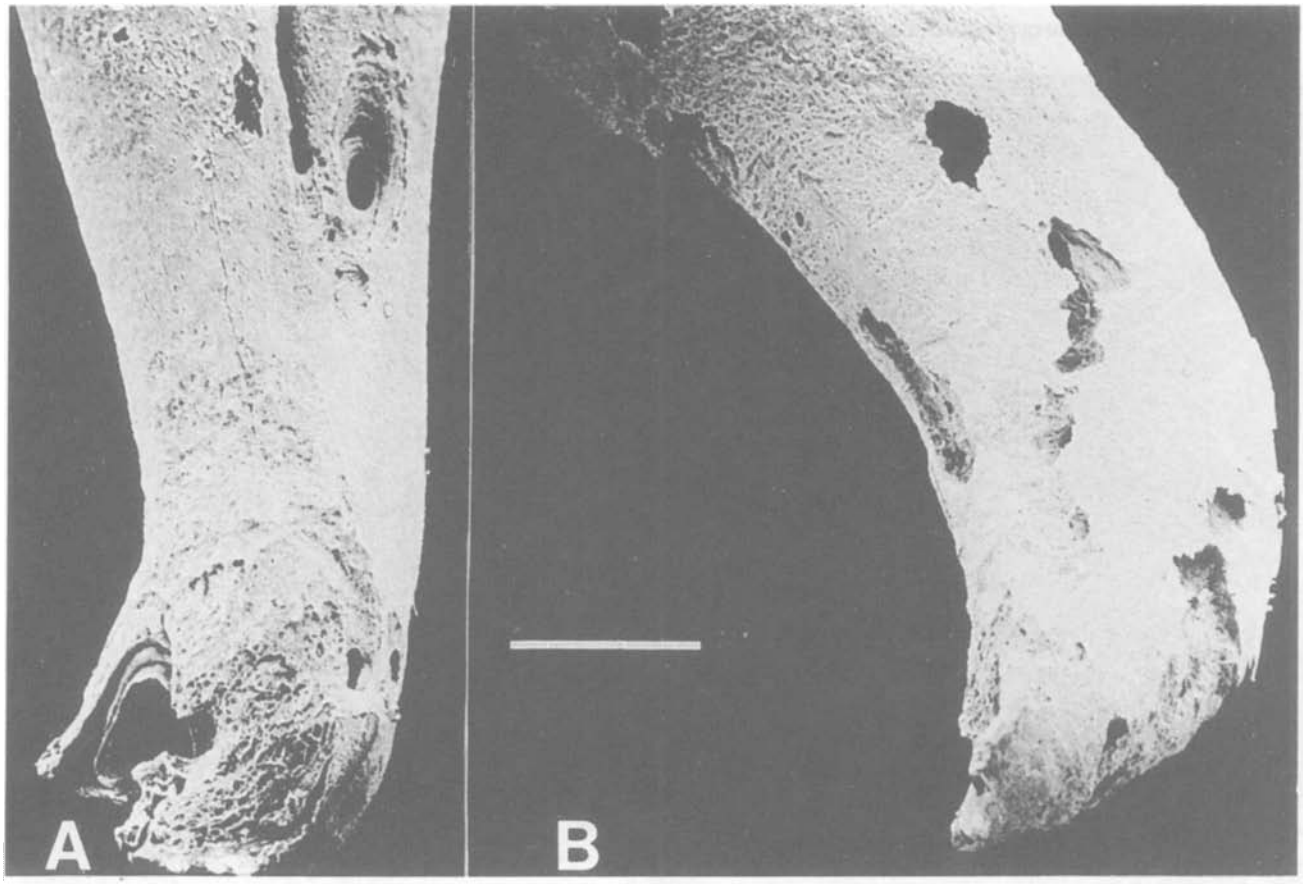

FIG. 2
A: Left incus, male, age 6 yrs., retraction pocket. Erosion of lenticular process. B: Left incus, male, age 5 yrs., cholestestoma. Erosion of lenticular and long processes. Scale bar = 500 microns.

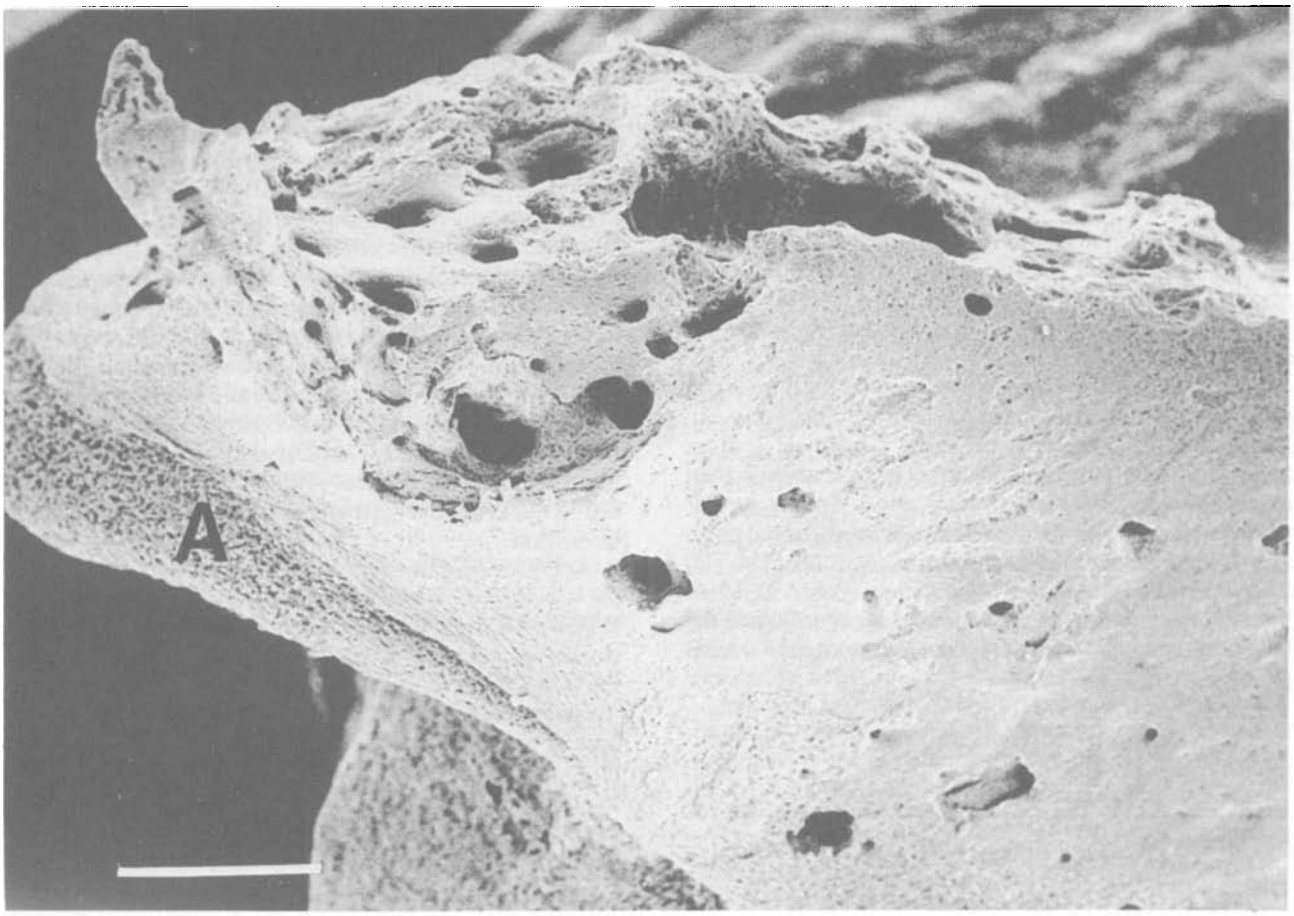

FIG. 3
Right incus, male, age 23 yrs., tubotympanic CSOM. Total erosion of long process, with extension onto body. Elsewhere there is 'pitting' of body and short process. A = articular surface, note characteristic surface texture. Scale bar = 500 microns.

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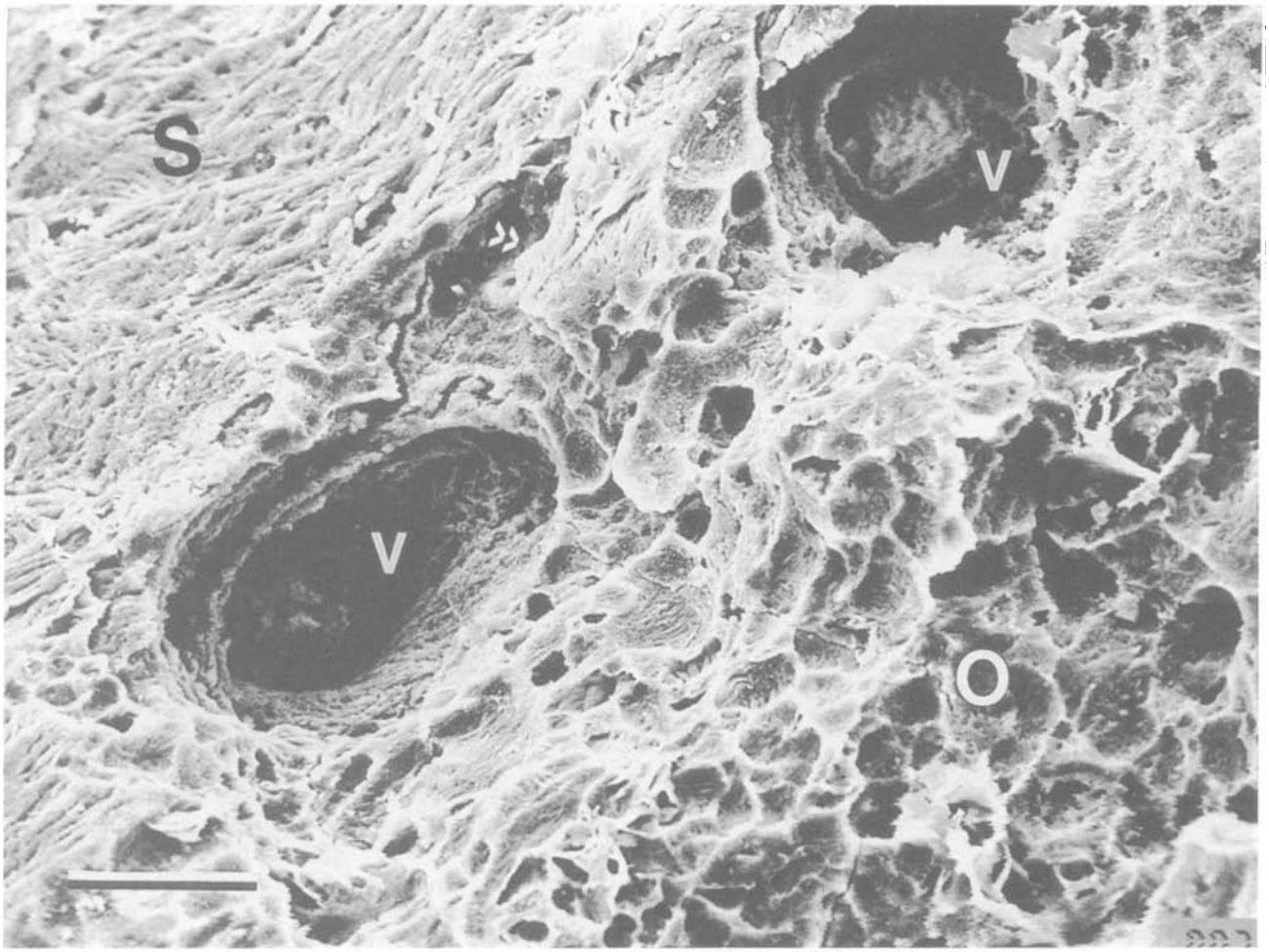

FIG. 4

Edge of erosive area. S = stable bone; O = osteoclasis. Some limited new bone formation along interface (arrowed). V = vascular foramen. Scale bar = 50 microns.

characterized by incomplete mineralization and rougher texture, in the areas of erosion (Fig. 1).

Discussion

The inability to identify osteoclasts in previous studies gave rise to other theories regarding the cellular mechanisms of bone erosion in CSOM. Chole (1988) suggests that the failure to identify osteoclasts may result from the practice of controlling inflammation with antibiotic and corticosteroid preparations prior to tympanomastoid surgery. However, there is no hard evidence to support this theory. The morphology of the osteoclast makes it difficult to identify on serial sections. It is a large multinucleated cell with branching processes and numerous microprojections. This means that only a small part of a single cell may be included in any section.

Other studies have demonstrated that an actively resorbing osteoclast may, in part, be separated from the bone surface by an interposing layer of cells (Jones and Boyde, 1977). This may further compound the identification of osteoclasts on serial sections.

By contrast, the T principle of SEM facilitates the recognition of sites of osteoclastic activity. It does so, not by identifications of the cell itself, but by the characteristic SEM appearances of Howship's lacunae (Jones and Boyde, 1977). Essentially, one is observing osteoclastic 'footprints'. This limits the conclusions that can be drawn from examination of a single specimen as it is not possible to comment on when osteoclastic resorption occurred, only that it did occur.

As with other studies (Grippaudo, 1958; Tos, 1979), the lenticular and long processes were the sites of greatest erosion in this series and it was not possible to determine the exact nature of the disease by the degree of erosion of the incus. Regardless of the disease type, all the areas of erosion were consistent with the activity of osteoclasts. No evidence was found to support any other proposed mechanism of bone resorption.

In conclusion, this study suggests that ossicular erosion in CSOM is due to osteoclastic activity in all the cases studied.

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