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Does occurrence of keratinizing stratified squamous epithelium in the middle-ear cavity always indicate a cholesteatoma?

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Abstract

The origin and behaviour of keratinizing stratified squamous epithelium, an essential component of cholesteatoma occurring in the middle-ear cavity, has puzzled otologists for decades. In this experimental study in 16 cats, central (n = 23) and peripheral (n = 9) tympanic membrane perforations were observed for up to 63 days before sacrifice. The tympanic membranes with bony rim were excised, decalcified and embedded in Epon 812. Sections were stained with toluidine blue and examined using a light microscope. The perforation had been sealed by meatal epithelium exhibiting pronounced hyperplasia and keratin formation, lying on a bed of granulation tissue. Subtotal central perforations healed within 14 days, forming a bowl-shaped tympanic membrane and leaving parts of the handle of the malleus (with meatal epithelium) protruding freely into the middle-ear cavity. Stratified squamous epithelium, morphologically identical with that of external ear canal epidermis, could be observed on the malleus even 63 days after operation. This meatal epithelium was non-keratinizing, non-invasive, and showed no destructive properties typical of acquired cholesteatoma. During certain circumstances, the cell cycle of hyperplastic epidermal epithelium within the middle-ear cavity can evidently be arrested and inactivated by a local defence mechanism.

Key words: Tympanic Membrane; Cholesteatoma; Animals, Laboratory; Ear Diseases; Wound Healing

Introduction

The aetiopathogenesis of cholesteatoma has puzzled otologists for decades. The patho-anatomical definition of a cholesteatoma is well established, *viz*. occurrence of keratinizing, stratified, squamous epithelium within the middle-ear cavity where otherwise only modified respiratory epithelium ought to be present. The epidermal epithelium of the cholesteatoma, originating mostly from the ear-drum, exhibits signs of hyperproliferation with migratory, invasive, and bone-destroying properties. Frequently, cholesteatomas form a pearl-like structure (matrix) composed of desquamated epithelial cells and keratin and surrounded by granulation tissue (perimatrix). Typically, cholesteatomas are associated with middle-ear infection accompanied by aural discharge.[1,2]

The healing process of a traumatic tympanic membrane perforation exhibits several features in common with a growing cholesteatoma: proliferation of the meatal squamous epithelium of the tympanic membrane accompanied by abundant production of keratin and granulation tissue.[3,4] When a small drum perforation heals, the tympanic membrane regains its original conical shape and its three-layered architecture (outer meatal keratinizing stratified squamous epithelium, inner tympanal epithelium in a single flat layer and, sandwiched in between, a connective tissue layer of mainly collagen fibres). However, after healing of a subtotal central perforation the original conical shape of the pars tensa is lost, and part of the malleus handle (with remnants of meatal epithelium) can protrude freely into the middle-ear cavity.[5,6] In such cases there is keratinizing, stratified, squamous epithelium within the middle-ear cavity or, according to the characterization stated above, a cholesteatoma.

The purpose of the present study was to observe over a period of weeks the appearance of keratinizing, stratified, squamous epithelium on the tympanic membrane following subtotal central and peripheral pars tensa perforations. Particular attention was paid to the meatal epithelium on the malleus handle, which was located within the middle-ear cavity, simultaneously with healing of a subtotal, central, traumatic perforation of the pars tensa. The cat, whose tympanic membrane is morphologically well described,[7] was chosen as the experimental animal.

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TABLE I

EXPERIMENTAL ANIMALS

Animal (No.)	Sex (M/F)	Weight (g)	Type of perforation Dx(%)	Sin(%)	Duration (days)
462	M	2400	C(90)	C(95)	2
463	F	1050	C(85)	C(80)	4
459	M	3800	C(80)	C(85)	4
453	M	4100	P(30)	C(50)	7
464	M	1720	C(60)	C(65)	7
460	M	3400	C(70)	C(60)	9
461	M	3900	C(65)	C(50)	9
465	M	1720	C(55)	C(30)	11
467	F	1080	C(0)	P(40)	14
468	M	1380	C(4)	P(5)	14
469	M	1300	P(0)	C(0)	28
454	F	2370	P(0)	C(0)	36
455	M	3250	P(0)	C(2)	37
456	F	2700	P(0)	C(5)	37
457	M	3100	P(0)	C(0)	63
458	M	3100	P(0)	C(0)	63

Number used, sex (M = male, F = female); their weight, type of perforation (C = central, P = peripheral; Dx = right, Sin = left; % = estimated size of remaining perforation at the end of experiment) and duration of experiment

Material and methods

A total of 32 tympanic membrane specimens from 16 cats were studied. The experimental animals constituted European mixed race cats of either sex (four female, 12 male), four to 24-months-old and with body weights ranging between 1050 and 4100 g (Table I). After a one-month quarantine, the animals were anaesthetized by intraperitoneal injection of Mebumal vet^{R} (ACO Inc., Sweden) 30 mg/kg body weight. After a retro-ventro auricular incision, the joint between the concha and the cartilaginous portion of the external auditory canal was split to visualize the tympanic membrane. None of the animals suffered from either external or middle-ear infection. Either a kidney-shaped perforation in all four quadrants (central perforation, n = 23) or a perforation in both rear quadrants of the drum (peripheral perforation, n = 9) was performed in the pars tensa with a myringotomy lancet and using a Zeiss otomicroscope. The annular ring of each rear quadrant was removed with a curette when a peripheral perforation was made (Figure 1).

After various intervals, ranging from two to 63 days, the animals were re-anaesthetized and the tympanic membrane examined and photodocumented. The animals were then killed with an overdose of Mebumal, after which they were decapitated, the temporal bones excised and fixed in ice-cold Karnovsky solution (paraformaldehyde-glutaraldehyde 1:1) for 24–48 hours. The temporal bones were trimmed and the tympanic membrane with its bony rim excised. The specimens were rinsed in buffer and transferred to 10 per cent EDTA. Decalcification was generally completed within 10–14 days, after which the sections were dehydrated in graded ethanol solutions and embedded in Epon 812. Semi-thin sections (1 μm), cut on a LKB ultratome, were stained with toluidine blue and then scrutinized under a Zeiss microscope and photodocumented.

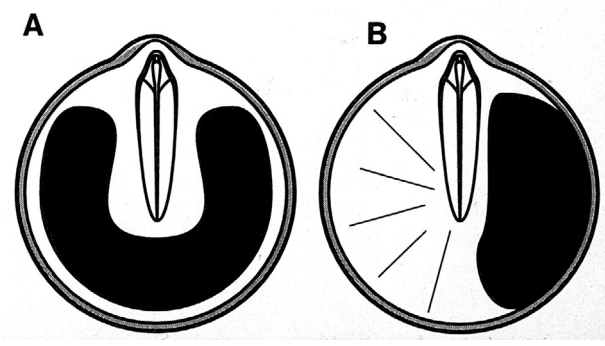

FIG. 1

Schematic drawings showing the experimental design. (a) Subtotal, central perforation and (b) peripheral drum perforation.

Results

Macroscopic findings

The kidney-shaped central perforation pierced all four quadrants of the pars tensa, leaving the annulus fibrosus intact. The entire handle of the malleus had a rim of tympanic membrane tissue (Figure 1(a)). No signs of infection either of the external auditory canal or of the tympanic cavity were observed in any animal.

The central perforations (Table I) had shrunk during the first two to four days (n = 6). Closure of the tympanal defect advanced mainly from the periphery and after seven to nine days approximately 40 per cent of the perforation was sealed (n = 7). The remaining defect was mainly located halfway between the annular ring and umbo in the lower quadrants of the pars tensa. Some 95–100 per cent sealing of the defect was established within 14 days (n = 2). After 28–37 days (n = 4) as well as after 63 days (n = 2) the scar tissue of the healed tympanic membrane was appreciably thickened. Two drums still harboured a tiny perforation. The original conical shape of the pars tensa was not re-established, and about two thirds of the malleus handle protruded freely into the middle-

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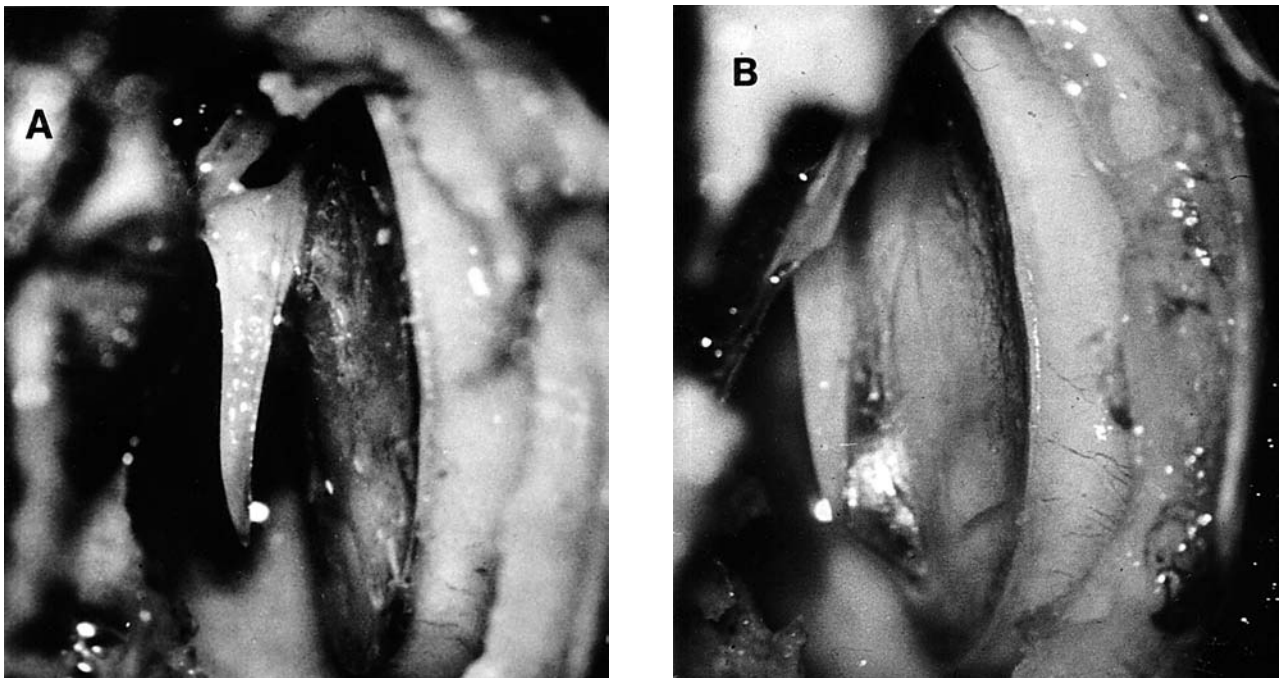

FIG. 2
Photographs viewing the tympanal side of the drum. (a) Healed central perforation with the handle of the malleus protruding into the middle-ear cavity (X6). (b) Healed peripheral perforation showing conically shaped pars tensa (X7).

ear cavity (Figure 2(a)). No visible meatal epithelium could be discerned on the malleus handle, which was completely covered with tympanal mucosa.

The peripheral perforations, occupying the two rear quadrants of the pars tensa in addition to the corresponding annulus fibrosus, shrunk considerably within 14 days (n = 3). Complete sealing of the tympanic membrane defect was noted after 28 days (n = 1), 36–37 days (n = 3) and 63 days (n = 2). The healed surface of the drum was visibly thickened. The original conical shape of the tympanic membrane was re-established (Figure 2(b)).

In contrast to a subtotal, central perforation, a peripheral perforation established contact between tissue originating from the tympanal rim and the handle of the malleus. No ingrowth of the ear-drum into the middle-ear cavity could be seen.

Microscopic findings

The orifice of the central perforation was reduced mainly by tissue advancing from the periphery of the tympanic membrane adjacent to annulus fibrosus. After only two to four days, pronounced epithelial proliferation was observed adjacent to the perforation rim which was markedly thickened, giving the appearance of a snake's head. The meatal epithelial layer reached 20–25 cells in thickness. In the central part of the 'snake's head', collagen fibres were seen together with granulation tissue. An extensive accumulation of keratin draped the 'snake's head', forming a long keratin spur in the direction of healing. The healing tissue was accompanied by round cells. The tympanal epithelium showed minimal, if any, morphological alterations and still had a flat appearance. The mucocutaneous junction between the meatal and tympanal epithelia was clearly located on the tympanal side of the tympanic membrane (Figure 3).

The tympanic membrane remnants of the handle of malleus were coiled on the meatal side of the handle. The collagen layer, together with the tympanal epithelium, appeared to enclose the meatal epithelium. Hyperplastic meatal epithelium with keratin flakes could be discerned within this tissue mass, which harboured many round cells. Clusters of mast cells were noted adjacent to vessels. The proliferation of the meatal epithelium was not as noticeable as was the corresponding epithelium at the annular rim.

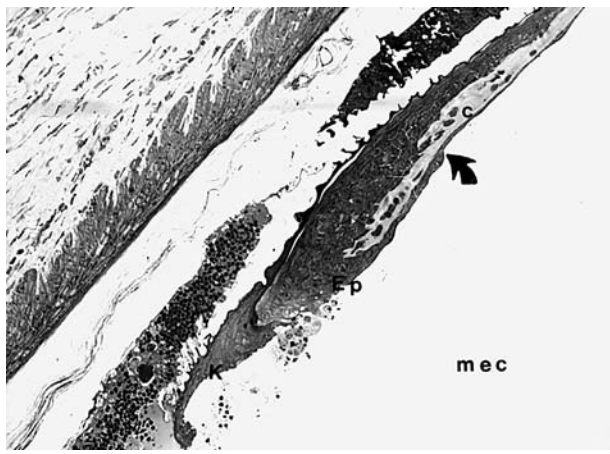

FIG. 3
Light micrograph (LM) of four-day-old central perforation (No. 459 dx), showing perforation rim with hyperplastic meatal epithelium (Ep), keratin spur (k), whorled collagen (c), mucocutaneous junction (arrow) and middle ear cavity (mec) (toluidine blue; X20).

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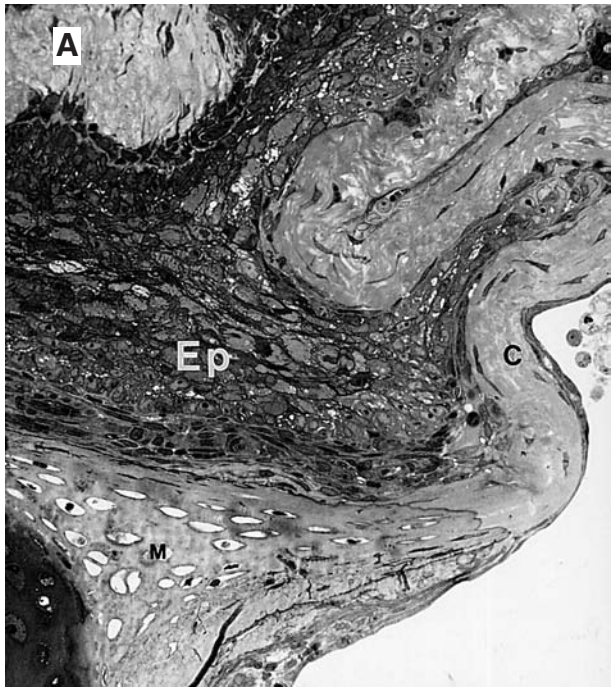

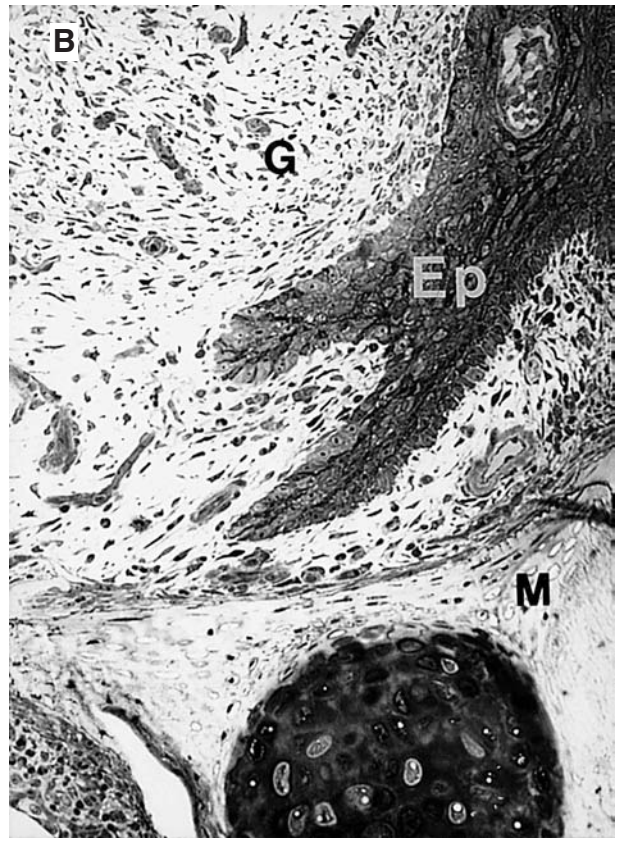

FIG. 4 (a) AND (b)
LMs of seven-day-old central perforation (No. 453 sin) showing section of handle of malleus (m) with hyperplastic meatal epithelium (Ep) embedded in collagen (c) and granulation tissue (G) (toluidine blue: X20).

After seven to nine days, about 40 per cent of the perforation orifice had been sealed by advancing tissue. Hyperplastic meatal epithelium with an advancing keratin spur was seen surrounding the perforation rim. The meatal epithelium was lying on a bed of granulation tissue.

Meatal epithelium was easily recognizable on the lateral side of the malleus handle. It was clearly hyperplastic and flakes of keratin were visible. Parts of the meatal epithelium were embedded in collagen, granulation tissue with round cells and tympanal epithelium (Figure 4 (a), (b)). In places, the meatal epithelium was shaped like rete pegs.

After 14 days, the central perforations were almost sealed. The healed drums exhibited a meatal epithelium whose hyperplasia had diminished to almost normal thickness, whereas the lamina propria was thickened. One perforation studied still had a small defect occupying four to five per cent of the original perforation in the lower part of pars tensa.

After 28 days, the meatal epithelium on the handle of malleus was still rather hyperplastic and keratin was still visible. In addition, organized connective tissue and granulation tissue with numerous round cells could be discerned between the malleus handle and the healed drum (Figure 5).

After 36–37 days the healed tympanic membrane was still thickened due to fibrotic thickening of the lamina propria. The meatal epithelium was almost of normal thickness. Minimal keratin formation was seen on the healed areas.

The meatal epithelium on the malleus was clearly visible and surrounded by richly vascularized connective tissue. The epithelium was flat but still showed signs of hyperplasia (Figure 6).

After 63 days the healed drum was still somewhat thickened due to a thick layer of lamina propria. The meatal and tympanal epithelia appeared normal.

A flat layer of meatal epithelial cells without keratin formation was noted within fibrotic connective tissue on the malleus. No signs of proliferation of meatal epithelium could be discerned (Figure 7).

The healing pattern of the peripheral drum perforations was similar to that described for the central ones: rapid epithelial cell proliferation with excessive keratin formation lying on a granulation tissue layer infiltrated with round cells. There was little, if any, hyperplasia of the tympanal epithelium. Encroachment of the meatal epithelium into the MEC was not observed in any of the specimens (Figure 8). All tympanic membrane defects in the peripheral perforations studied had been sealed, 28 days and onwards, after which the meatal epithelium had regained normal thickness.

Discussion

The present study elucidated the healing mechanism of a traumatic tympanic membrane perforation, i.e. the orifice was initially sealed by hyperplastic meatal epithelium, which showed profuse keratin production and located on a bed of granulation tissue. Subsequently, the epithelial hyperplasia and keratin formation in the perforation area were quickly (within one or two days) reduced, whereas the granulation tissue already formed was transformed into connective

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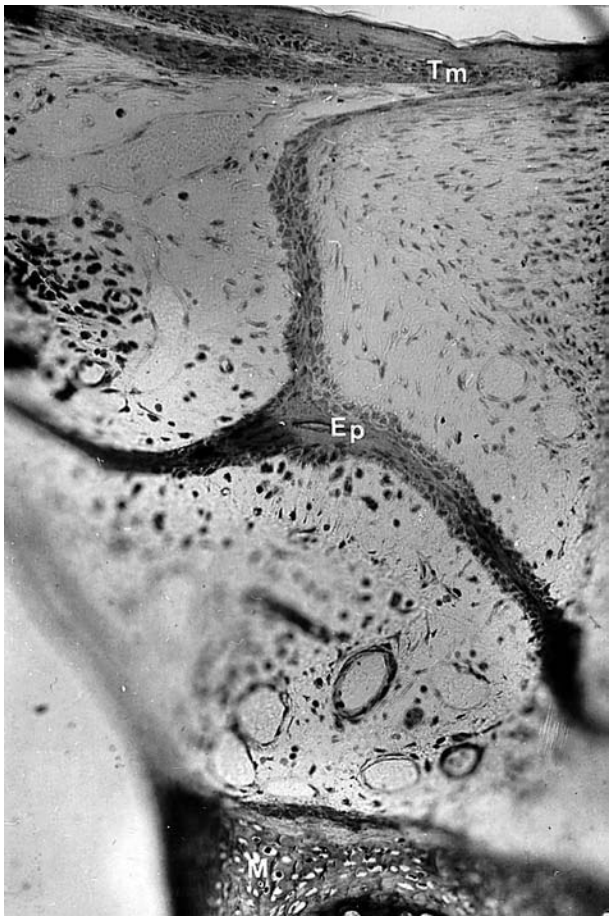

FIG. 5

LM of 28-day-old perforation (No. 469 sin) showing a healed drum (Tm) and meatal epithelium (Ep) embedded in connective tissue. Handle of malleus (M) (toluidine blue X20).

tissue (over weeks). This caused the healed tympanic membrane to be thicker than originally. No encroachment of meatal epithelium into the middle-ear cavity was seen following a peripheral perforation.

The prolific meatal epithelial hyperplasia with profuse keratin and granulation tissue formation is typical of an acquired middle-ear cholesteatoma.[1,2] A remarkable difference between tympanic membrane healing and cholesteatoma is that in the latter the epithelial hyperplasia, keratin formation and granulation tissue formation are continuous processes that do not subside.

Permanent epithelial hyperplasia of the tympanic membrane can also occur if the healing process is blocked by inserting a piece of plastic or ventilation tube in the tympanic membrane.[8] In such cases the granulation tissue formation in the tympanic membrane is transformed into connective tissue with a few round cells. Keratin formation in the area still proceeds. When the ventilation tube is removed, the tympanic membrane will heal quickly as described above.

Epidermal remnants in the middle-ear cavity may be congenital. These formations, some of which produce keratin, are supposed to be spontaneously eliminated.[9] Whether or not these epidermal remnants can develop into an unmistakable

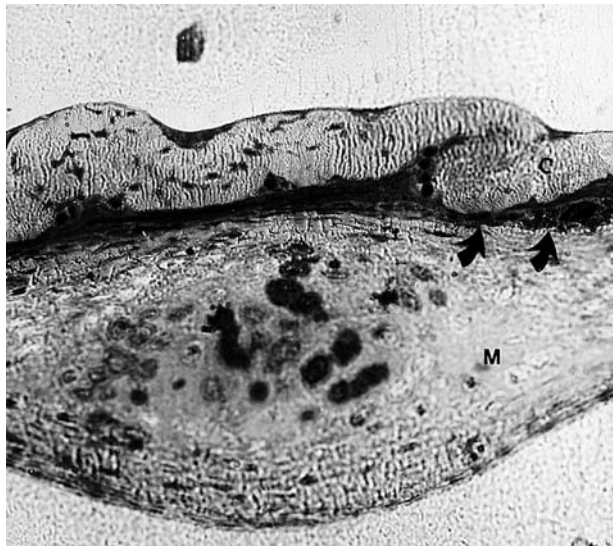

FIG. 6

Handle of malleus (m) 37 days after central perforation (No. 455 sin) showing meatal epithelium (arrows) completely embedded in collagen (c) and thin tympanal epithelium (toluidine blue; X20).

cholesteatoma has not been established.[10] A prerequisite for keratin production is the presence of stratum granulosum in the epithelium. Any epithelium containing a granular cell layer within the middle-ear cavity will continue to produce keratin and subsequently form a cholesteatoma.[11] In the present study, meatal epithelium in the middle-ear cavity could be discerned for at least 63 days and elimination of these cells seemed very tardy. If the meatal epithelium is eliminated by apoptosis, genetically encoded cell death, then the eliminating process ought to be faster. It seems more likely that the cell cycle of the meatal epithelial cells is arrested than that they are eliminated by apoptosis, which include a rapid phagocytosis of cell remnants. Such possibilities were also suggested in recent studies on human middle-ear cholesteatoma.[12,13]

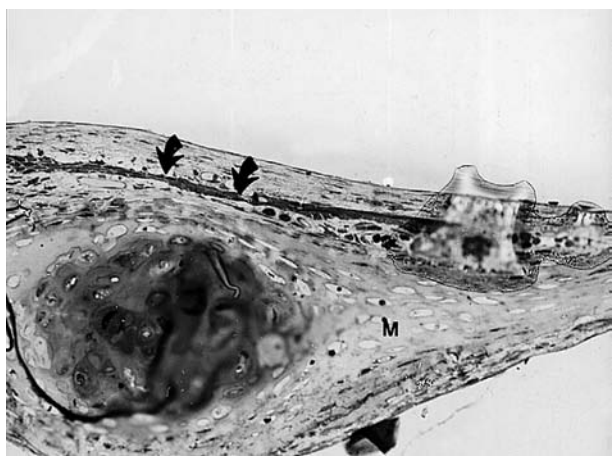

FIG. 7

Handle of malleus (M) 63 days after central perforation (No. 458 sin) with remnants of meatal epithelium (arrows) without keratin formation, completely embedded in connective tissue (toluidine blue; X20).

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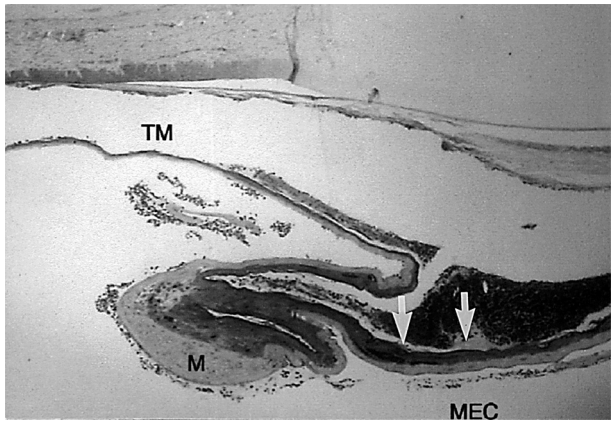

FIG. 8

LM of 14-day-old peripheral perforation (No. 468 sin). Malleus (M). Unaffected anterior quadrants (TM). Healed drum area (arrows). Middle-ear cavity (MEC). Note the hyperplastic epithelium, perforation not completely sealed (toluidine blue; X20).

- **The origin and behaviour of keratinizing stratified squamous epithelium, an essential component of cholesteatoma occurring in the middle-ear cavity (MEC), has puzzled otologists for decades**
- **In this experimental study in 16 cats central and peripheral tympanic membrane perforations were observed for up to 63 days before sacrifice**
- **Subtotal central perforations healed within 14 days. The perforation gap had been sealed by meatal epithelium exhibiting pronounced hyperplasia and keratin formation, lying on a bed of granulation tissue**
- **Stratified squamous epithelium, which was non-keratinizing, non-invasive and showed no destructive properties typical of cholesteatoma and which was found to be morphologically identical with that of the external ear canal epidermis, was observed on the malleus even at 63 days post-operation**
- **The authors conclude that during certain circumstances, the cell cycle of hyperplastic epidermal epithelium within the MEC can evidently be arrested and inactivated by a local defence mechanism**

An important event in the aetiopathogenesis of acquired cholesteatoma seems to be the protracted hyperplasia of the epidermal epithelium. This process may be maintained by genetic factors located in the epithelial cells[13] or by external stimuli in the form of cytokines released by, for instance, infiltrating inflammatory cells.[14,15] Hyperplasia of the epidermal epithelium on its own, however, cannot explain the development of cholesteatoma. Thus, it seems that an exogenous irritative factor must also be present in the middle-ear cavity. One such factor could well be persistent middle-ear infection.[1,2]

It is well-established that the tympanic membrane, particularly its meatal epithelium, is in one way or another involved in the aetiopathogenesis of most middle-ear cholesteatomas.[2] This article presents a valuable experimental model with which to study the behaviour of epidermal epithelium in the middle-ear cavity. Although non-infectious subtotal central tympanic membrane perforations are relatively rare in the clinic, they may occur in connection with blast injuries or direct trauma to the tympanic membrane.[16] The present model can be added to other experimental models, e.g. transfer of skin flaps from the external auditory meatus into the middle-ear cavity[17] or insertion of a gelatin membrane through the tympanic membrane in order to induce ingrowth of meatal epithelium into the middle-ear cavity.[18,19]

The present study revealed several biologically and clinically important events showing how the middle-ear cavity reacts to trauma. Sealing of a large tympanic membrane defect seemed to be more important than the restoration of good hearing. Sealing of a subtotal perforation was established within two weeks, but the original conical shape of the pars tensa was not regained. The healed tympanic membrane was remarkably flattened, almost bowl-shaped, and the distal part of the malleus handle protruded freely into the middle-ear cavity, scarcely contacting pars tensa (Figure 2(a)). This circumstance naturally affected the transduction of sound energy from the tympanic membrane to the ossicles. Moreover, it offered an evident risk of implanting meatal epithelium into the middle-ear cavity. Such implanted epithelium was, in this case, a finger-like extension of the meatal epithelium of tympanic membrane.

Signs of healing of the tympanic membrane were visible along the whole perforation rim. Extensive hyperplasia of the meatal epithelium was noted close to the perforation gap where the epithelial cell layer numbered 20–25 cells in thickness (Figure 3) compared to two to three cells of a normal, untouched tympanic membrane.[7] The epithelium produced keratin abundantly, especially along the rim of the perforation. In fact, the abundant keratin produced formed a disc that seemed to guide the healing process in the right direction.

On the handle of the malleus, the meatal epithelium was also hyperplastic, but keratin production was not as abundant as along the perforation rim. Although the meatal epithelium along the handle of malleus did not participate in sealing subtotal central tympanic membrane defects to any great extent, epithelial hyperplasia could be discerned at least as long as the perforation was patent, i.e. 14 days, after which it seemed to cease and the meatal epithelium became completely enveloped by connective tissue. However, epithelium morphologically identical with that of the external ear canal epidermis was still clearly visible on day 63 when the tympanic membrane perforation had been sealed for weeks. This epidermal epithelium was non-keratinizing, non-invasive and showed minimal, if any, activity (Figure 7). Thus, it seems that the proliferation of the meatal epithelium of the tympanic membrane is strictly controlled and the proliferative

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state can be switched on and off. Whether or not this epidermal epithelium present in the middle-ear cavity has the potential to become hyperplastic and keratinizing again requires further study.

Ear surgeons have observed that keratinizing, stratified, squamous epithelium left behind in an inaccessible area of the middle-ear cavity during a cholesteatoma operation can sometimes disappear spontaneously. This finding can be confirmed at a 'second-stage operation'. However, it might be that such epidermal epithelium is only transformed into a non-keratinizing, non-invasive one which starts to proliferate for instance in connection with a middle ear infection.[20] It is well known that cholesteatomas grow in combination with drainage from the external auditory meatus. It cannot be excluded that bacteria present in the external auditory canal are of particular importance in this connection. The two bacterial species most frequently found in connection with a draining cholesteatoma are *Staphylococcus aureus* and *Pseudomonas aeruginosa*,[21, 22] both of which can inhabit the healthy external auditory canal.[23, 24] It is worth mentioning that any middle-ear operation can imply a risk of introducing these pathogens into the middle-ear cavity.

The only truly effective treatment for middle-ear cholesteatoma currently available is surgery.[2] The present study suggests that it might be possible to deal with invaded stratified squamous epithelium in the middle ear by applying means other than surgery, provided that the defence mechanisms are the correct ones and strong enough. Whether or not control of middle-ear infection, or prevention of granulation tissue formation in the middle-ear cavity, is enough in this respect calls for further experimental studies.

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References

1 Albino AP, Kimmelman CP, Parisier SC. Cholesteatoma: a molecular and cellular puzzle. *Am J Otol* 1998;**19**:7–19
2 Olsewska E, Wagner M, Bernal-Sprekelsen M, Ebmeyer J, Dazert S, Hildmann H, *et al*. Etiopathogenesis of cholesteatoma. *Eur Arch Oto-Rhino-Laryngol* 2003; published 'online' 27 June
3 Reeve DRE. Repair of large experimental perforations of the tympanic membrane. *J Laryngol Otol* 1977; **91**:767–78
4 Stenfors L-E. Structure and pattern of motion of the tympanic membrane with special reference to experimental perforations and subsequent healing processes. Umeå University Medical Dissertations No 5. Karleby, Finland: Österbottningens tryckeri, 1980
5 McIntire C, Benitez JT. Spontaneous repair of the tympanic membrane. Histopathological studies in the cat. *Ann Otol Rhinol Laryngol* 1979;**6**:112–31
6 Stenfors L-E, Carlsöö B, Salén B, Winblad B. Repair of experimental tympanic membrane perforations. *Acta Otolaryngol* 1980; **90**:332–41
7 Lim DJ. Tympanic membrane. Electron microscopic observation. Part I: Pars tensa. *Acta Otolaryngol* 1968; **66**:181–98
8 Stenfors L-E, Winblad B. Tympanic membrane changes after instillation of polyethylene grommets. *Acta Otolaryngol* 1980;**90**:16–24
9 Michaels L. Origin of congenital cholesteatoma from a normally occurring epidermoid rest in the developing ear. *Int J Pediatr Otorhinolaryngol* 1988;**15**:51–65
10 Levine JL, Wright CG, Pawlowsk KS, Meyerhoff WL. Postnatal persistence of epidermoid rests in the human middle ear. *Laryngoscope* 1998;**108**: 70–3
11 Palva T, Makinen J. Why does middle ear cholesteatoma recur? *Arch Otolaryngol* 1983;**109**:513–8
12 Kojima H, Tanaka Y, Tanaka T, Miyazaki H, Shiwa M, Kamide Y, *et al*. Cell proliferation and apoptosis in human middle ear cholesteatoma. *Arch Otolaryngol Head Neck Surg* 1998;**124**:261–4
13 Huisman MA, De Heer E, Grote JJ. Cholesteatoma epithelium is characterized by increased expression of Ki-67, p53 and p21, with minimal apoptosis. *Acta Otolaryngol* 2003;**123**:377–82
14 Chung JW, Yoon TH. Different production of interleukin-1 alpha, interleukin-1 beta and interleukin-8 from cholesteatomous and normal epithelium. *Acta Otolaryngol* 1998;**118**:386–91
15 Myers EN, Park K, Chun YM, Lee DH, Hwang SC. Signal transduction pathway in human middle ear cholesteatoma. *Otolaryngol Head Neck Surg* 1999;**120**:899–904
16 Kringlebotn M. Rupture pressures of membranes in the ear. *Laryngoscope* 2000;**109**:940–4
17 Jackson DJ, Lim DJ. Fine morphology of the advancing front of cholesteatoma – experimental and human. *Acta Otolaryngol* 1978;**86**:71–88
18 Hueb MM, Goycoolea MV, Muchow D, Duvall AJ, Paparella MM, Sheridan C. In search of missing links in otology. III. Development of a new animal model for cholesteatoma. *Laryngoscope* 1993;**103**:774–84
19 Goycoolea MV, Hueb MM, Muchow D, Paparella MM. The theory of the trigger, the bridge and the transmigration in the pathogenesis of acquired cholesteatoma. *Acta Otolaryngol* 1999; **119**:244–8
20 Palva T. Histopathology is neglected in teaching of otology. *Int J Pediatr Otorhinolaryngol* 2003; **67:** 681–3
21 Vartiainen E, Vartiainen J. Effect of aerobic bacteriology on the clinical presentation and treatment results of chronic suppurative otitis media. *J Laryngol Otol* 1996; **110**:315–18
22 Silvola J, Palva T. Long-term results of pediatric primary one-stage cholesteatoma surgery. *Int J Pediatr Otorhinolaryngol* 1999;**48**:101–7
23 Stroman DW, Roland PS, Dohar J, Burt W. Microbiology of normal external auditory canal. *Laryngoscope* 2001; **111**:2054–9
24 Stenfors L-E, Räisänen S. Quantity of aerobic bacteria in the bony portion of the external auditory canal of children. *Int J Pediatr Oto-Rhino-Laryngol* 2002;**66**:167–73

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