The Journal of Laryngology and Otology
January 1996, Vol. **110**, pp. 13–18

Main Articles

Acute labyrinthitis associated with systemic *Candida albicans* infection in ageing mice

ROBERT B. ASHMAN, PH.D., JOHN M. PAPADIMITRIOU, M.D., PH.D., ALMA FULURIJA

Abstract

The yeast *Candida albicans* is an important opportunistic pathogen that has been associated with disease of the inner ear. This study describes the histopathology of acute labyrinthitis caused by systemic infection with *C. albicans* in ageing inbred mice. Within four days after infection, yeast and hyphal forms of *C. albicans* were found in the membranous labyrinth. The utricle and the adjacent parts of the ampullary regions of the semicircular canals were most severely affected, but damage was also seen in the scala media, the scala tympani, the saccule, and the scala vestibuli. In the utricle, the lining epithelium of the membranous labyrinth was disrupted, and the lining cells of the vestibular membrane showed foci in which the membrane was disrupted. The data suggest that age may represent a risk factor for fungal labyrinthitis.

Key words: *Candida albicans*; Labyrinthitis; Ageing

Introduction

In humans, labyrinthitis and sensorineural hearing loss are common complications of bacterial meningitis (Blank *et al.*, 1994), but otologic sequelae have also been reported as a consequence of infections with measles virus (Fukuda *et al.*, 1994) and cytomegalovirus (Rarey and Davis, 1993). Mycotic infections of the inner ear have been reported only rarely. A recent survey of the published literature identified nine cases of skull base osteomyelitis (Hanna *et al.*, 1993), most of which were associated with immunosuppression in the elderly. Causative agents included *Candida* species (Meyerhoff *et al.*, 1979), *Cryptococcus* (McGill, 1978), *Blastomyces* (Louis and Lockey, 1974), and *Mucor* (Bergestrom *et al.*, 1970; Gussen and Canalis, 1982).

Candidiasis is becoming increasingly prevalent in the hospital environment (Wade, 1993), and immunosuppressed or debilitated patients are particularly at risk. Systemic candidiasis in inbred mice reproduces many of the features of the human disease, in that the lesions are similar in nature and distribution (Papadimitriou and Ashman, 1986), and the brain and kidney are major foci of infection. However, CBA/CaH mice, that develop severe lesions in the brain and other tissues after systemic infection (Ashman and Papadimitriou, 1987), also exhibit a focal imflammation in the petrous temporal near the inner ear (Ashman and Papadimitriou, 1989), that persists for at least six months (Ashman and Papadimitriou, 1991). Because the elderly often show an increased susceptibility to infectious disease, we examined the effect of *Candida albicans* infection in aged mice, and here describe the invasion and rapid colonization of the membranous labyrinth of the inner ear.

Materials and methods

Specific-pathogen-free CBA/CaH mice were purchased from the Animal Resources Centre, Perth, Australia at six to eight weeks of age, and maintained in conventional animal facilities for a further 16 months. Only female mice were used in experiments. All animals were housed and used in accordance with the NH & MRC/CSIRO/Australian Agricultural Council's Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia, 1985. The experimental protocols were approved by the University of Western Australia Animal Ethics Committee.

Candida albicans isolate 3630 was obtained from the Mycology Reference Laboratory at the Royal North Shore Hospital, Sydney, and kept in small aliquots at −70 °C in 15 per cent glycerol in Sabouraud's broth. A loop of the frozen stock was inoculated into Sabouraud's broth, and grown for two days at room temperature, with constant

From the Department of Pathology, University of Western Australia, Nedlands, W.A. 6009. Australia.
Accepted for publication: 2 November 1995.

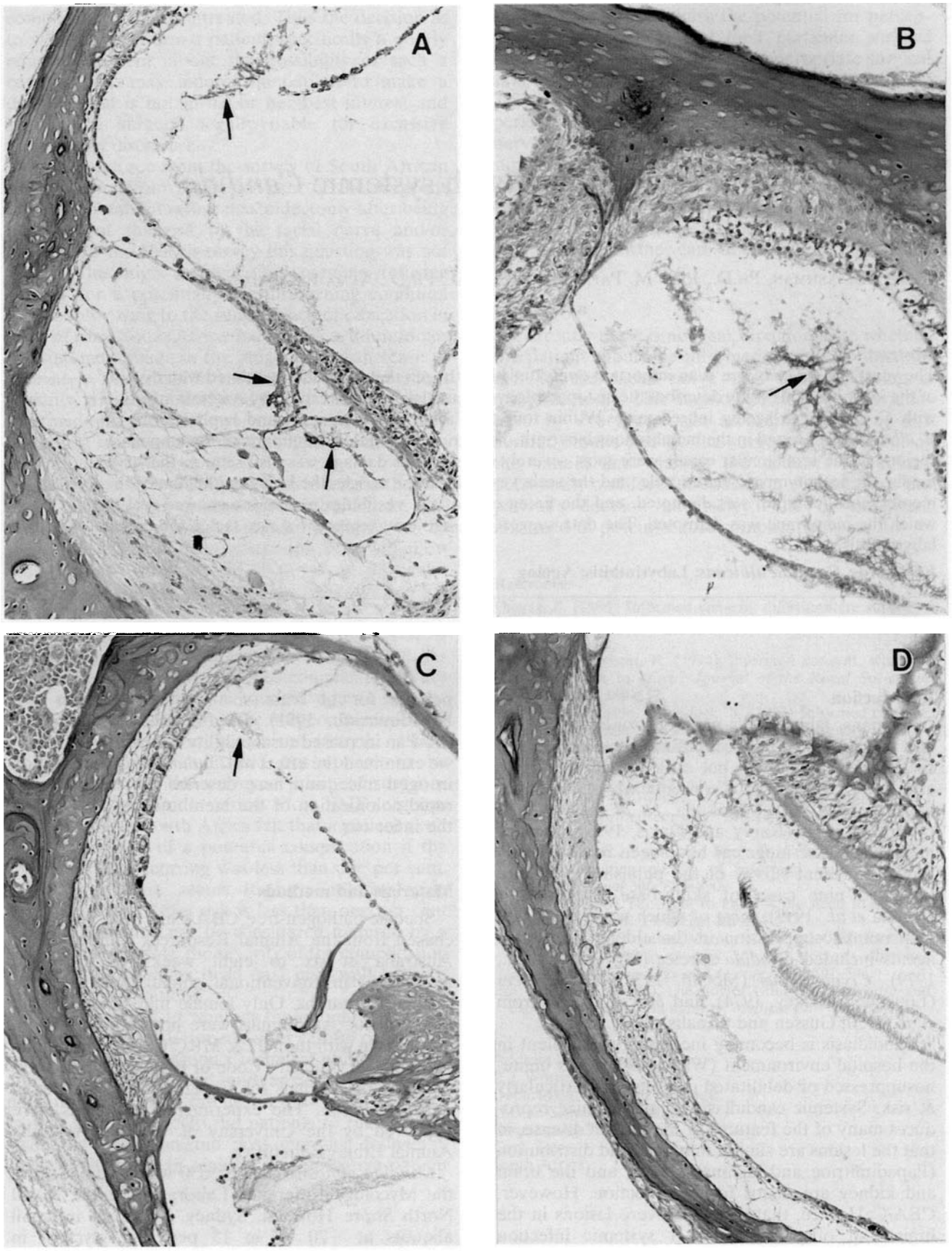

FIG. 1

Inner ear of a mouse four days after infection with 3×10^5 blastoconidia of *Candida albicans*. (a) Inflammatory exudates and cellular debris (arrows) are seen within the semicircular canals and utricle. (PAS; ×140). (b) Inflammatory exudate and cell debris (arrow) are present on the utricle in the vicinity of the neuroepithelium. (PAS; ×160). (c) Inflammatory exudates and cell debris are present in the cochlea, especially the scala media and scala tympani. Note the discontinuity of the lining epithelium (arrow). (PAS; ×140). (d) Inflammatory exudate and cell debris are seen in the saccule. (PAS; ×140).

https://doi.org/10.1017/S0022215100132621 Published online by Cambridge University Press

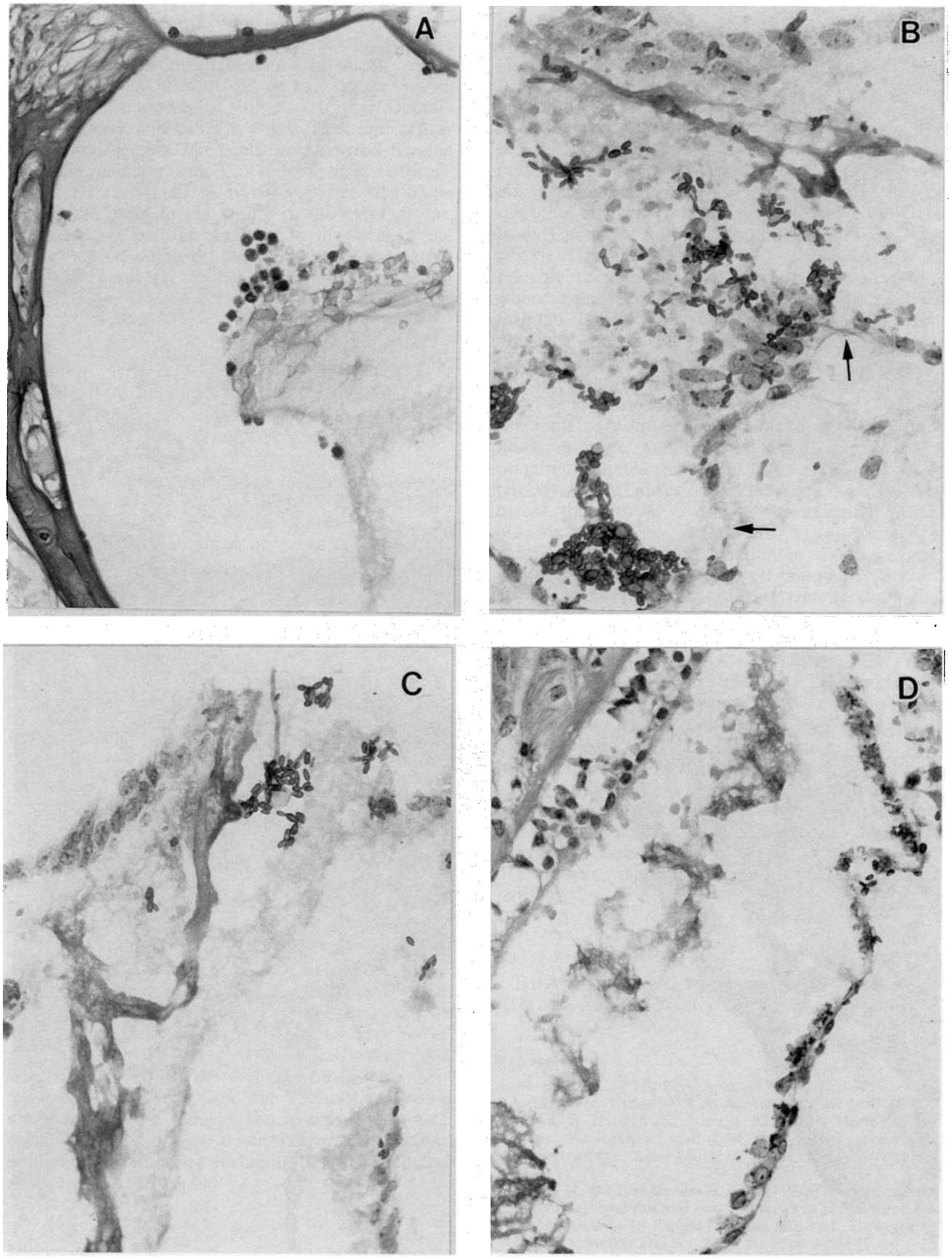

FIG. 2

Inner ear of a mouse four days after infection with 3×10^5 blastoconidia of *Candida albicans*. (a) Proteinaceous exudate and several mononuclear leucocytes in the scala tympani. (PAS; ×350). (b) Proteinaceous exudate, fibrin strands and many yeasts within the utricle. Note the disruption of the lining epithelium (arrows). (PAS; ×400). (c) Yeasts, mycelia, fibrin strands proteinaceous exudate in the vicinity of disrupted epithelium in the utricle. (PAS; ×400). (d) Inflammatory exudate near the neuroepithelium of the utricle, while yeasts have invaded the cells of the lining epithelium. (PAS; ×400).

https://doi.org/10.1017/S0022215100132621 Published online by Cambridge University Press

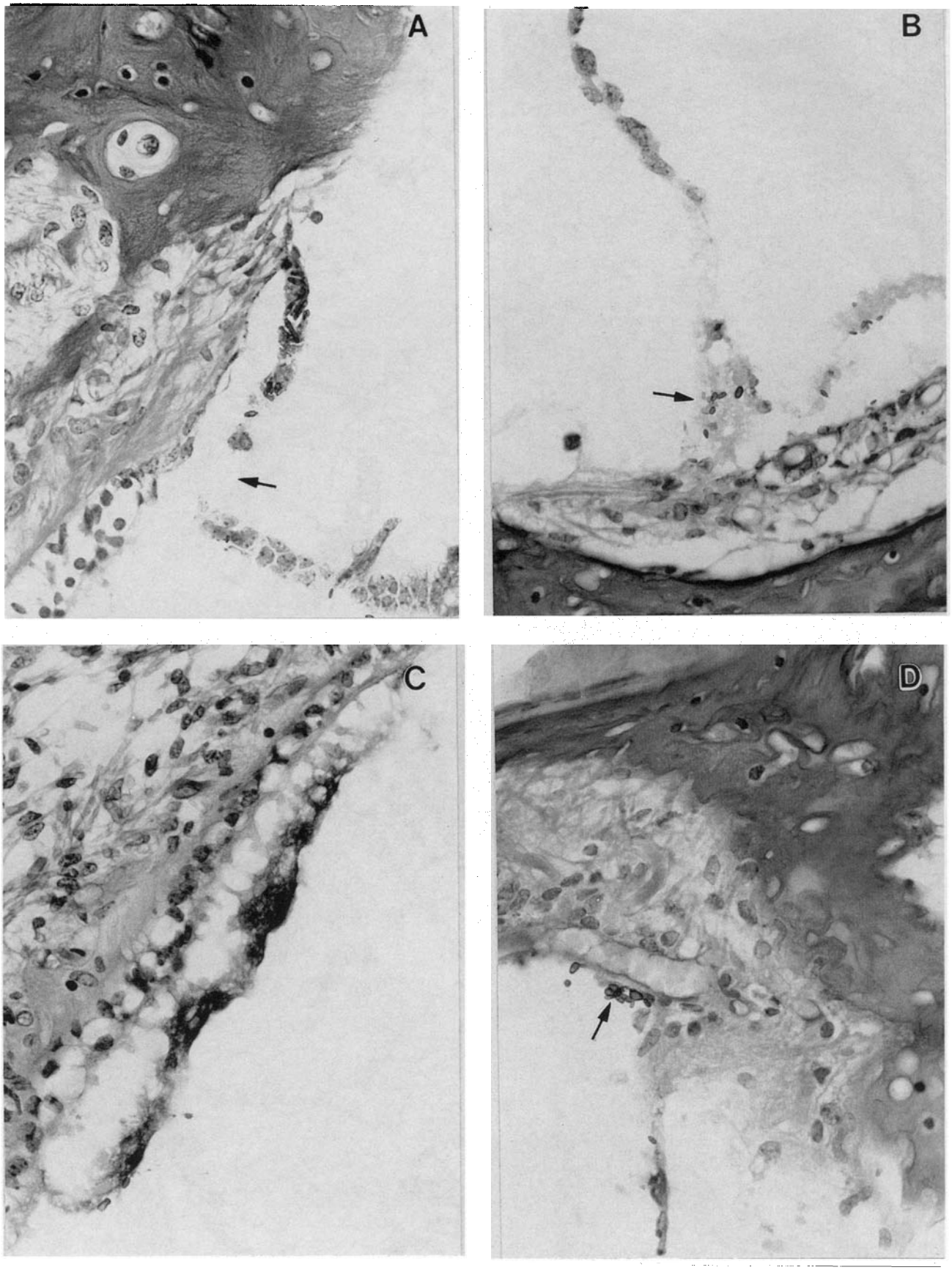

FIG. 3

Inner ear of a mouse four days after infection with 3×10^5 blastoconidia of *Candida albicans*. (a) Yeasts have invaded the lining epithelial cells of the utricle. Note the breaks in continuity (arrow). (PAS; ×400). (b) Necrotic epithelium in the scala tympani together with the presence of yeasts in the debris (arrow). (PAS; ×400). (c) Inflammatory exudate and cell debris abut on the neuroepithelium of the scala tympani. (PAS; ×400). (d) Blastospores (arrow) are present in the perivascular region of the periosteum. (PAS; ×450).

https://doi.org/10.1017/S0022215100132621 Published online by Cambridge University Press

agitation. Blastospores were washed in phosphate-buffered saline, and adjusted to the appropriate concentration for inoculation into the mice.

Two groups of mice, 10 old animals and five young controls, were inoculated with 3×10^5 blastoconidia intravenously, and killed by cervical dislocation four days after infection. The heads were severed, fixed in formalin, and decalcified in sodium formate/formic acid for three weeks. A transverse slice was taken across the brain in the region of the external ear, embedded in paraffin wax, cut to the level of the inner ear and serial sections stained with haematoxylin and eosin (H & E), or periodic acid-Schiff (PAS).

Results

Within four days after inoculation, the aged mice developed extreme vertigo and showed symptoms of severe distress. Several animals were close to death, and for ethical reasons, the experiment was terminated at this point. Infected control animals, six to eight weeks of age, displayed few, mild symptoms, with no morbidity.

Histological examination of the inner ear of the old animals revealed yeast and hyphal forms of *C. albicans* in the membranous labyrinth. The most affected regions, in order of severity, were the utricle and the adjacent parts of the ampullary regions of the semicircular canals, the scala media, the scala tympani, the saccule, and the scala vestibuli (Figures 1–3). Eosinophilic protein precipitates and fibrin strands were seen in all of these compartments (Figures 1–3), and in the scala tympani, they were often surrounded by aggregates of leucocytes (Figure 2a). In several instances these proteinaceous deposits abutted onto the neuroepithelium (Figure 3c). Blastospores, and less frequently, mycelia, were seen in the endolymphatic space of the utricle and scala media (Figures 2 and 3), and occasionally, a few organisms were observed in the near vicinity of the neuroepithelium (Figure 3b). In the utricle, the lining epithelium of the membranous labyrinth was disrupted (Figures 2b and c), while the lining cells were somewhat enlarged and displayed many granules in their cytoplasm. Blastospores abutted onto the lining epithelium, sometimes invading it (Figure 3a), and on some occasions they were present in the perilymphatic space within the vestibule, or within the periosteum (Figure 3d). Similarly, yeasts lay adjacent to the vestibular membrane, where some of the lining cells showed changes similar to those described for the utricular epithelium (Figure 3b), including foci in which the membrane was disrupted.

Discussion

The novel finding in the present experiments is that ageing mice develop an acute fungal labyrinthitis in association with disseminated candidiasis. The pathological patterns were similar to those described in autopsy studies of patients with meningogenic labyrinthitis (Blank *et al.*, 1994); and in experimental *Streptococcus pneumoniae* infections in rabbits (Bhatt *et al.*, 1991), and guinea pigs (Blank *et al.*, 1994). The lesions that were seen in the membranous labyrinth, including the presence of protein exudates, microorganisms and leucocytes, would disrupt labyrinth functions and result in the deafness previously described after sublethal *C. albicans* infection (Ashman and Papadimitriou, 1989), and in the other clinical symptoms displayed by CBA/CaH mice. The extact route of entry of the yeasts into the labyrinth has not been identified; however, it is possible that they entered the endolymph somewhere near its source, or spread to the cochlea through the perilymphatic duct because of the presence of meningoencephalitis. Alternatively, they may have invaded the labyrinth from a focus of osteomyelitis in the petrous temporal (Ashman and Papadimitriou, 1989).

Haematogenous and disseminated *Candida* infections are more common in patients with dysfunctional neutrophils (Kim *et al.*, 1969) or neutropenia (Bodey, 1966; Lehrer and Cline, 1971); and to this extent, the unusual severity of the infections in the old mice may be attributable to a decline in neutrophil numbers or function in these animals. However, specific cell-mediated immune responses are also required for efficient clearance of the organism (Ashman and Papadimitriou, 1990), and a gradual decline with age in numbers of *Candida*-specific memory T-lymphocytes (Ashman, 1982) may increase susceptibility to infection. Whatever the mechanism, the present finding in mice suggests that old age may *per se* increase the risk of unusual manifestations of *Candida* infections, and also indicates that the possibility of fungal labyrinthitis should be considered in elderly patients presenting with unexplained ear pain and evidence of inner ear disease. In addition, the CBA/CaH mouse strain may provide a useful model for the further study of hearing loss associated with inflammatory cochlear pathology.

Acknowledgments

RBA is supported by a grant from the Medical Research Fund of Western Australia.

References

Ashman, R. B. (1982) Persistence of cell-mediated immunity to influenza A virus in mice. *Immunology* **47:** 165–168.

Ashman, R. B., Papadimitriou, J. M. (1987) Murine candidiasis. Pathogenesis and host responses in genetically distinct inbred mice. *Immunology and Cell Biology* **65:** 163–171.

Ashman, R. B., Papadimitriou, J. M. (1989) Genetic regulation of pathogenesis and host responses in fungal infection. In *Immunology of Fungal Infection* (Kurstak, E., Marquis, G., eds.), Marcel Dekker, New York, pp 347–371.

Ashman, R. B., Papadimitriou, J. M. (1990) What's new in the mechanisms of host resistance to *Candida albicans* infection? *Pathology Research and Practice* **186:** 527–534.

Ashman, R. B., Papadimitriou, J. M. (1991) Chronic osteomyelitis as a consequence of systemic *Candida albicans* infection. *Immunology and Cell Biology* **69:** 427–429.

Bergestrom, L., Hemenway, W., Barnhart, R. (1970) Rhino-

cerebral and otologic mucormycosis. *Annals of Otology, Rhinology and Laryngology* **79:** 70–81.
Bhatt, S., Halpin, C., Hsu, W., Thedinger, B. A., Levine, R. A., Tuomanen, E., Nadol, J. B., Jr. (1991) Hearing loss and pneumococcal meningitis: an animal model. *Laryngoscope* **101:** 1285–1292.
Blank, A. L., Davis, G. L., Van De Water, T. R., Ruben, R. J. (1994) Acute *Streptococcus pneumoniae* meningogenic labyrinthitis. An experimental guinea pig model and literature review. *Archives of Otolaryngology–Head and Neck Surgery* **120:** 1342–1346.
Bodey, G. P. (1966) Fungal infections complicating acute leukemia. *Journal of Chronic Disease* **19:** 667–687.
Fukuda, S., Ishikawa, K., Inuyama, Y. (1994) Acute measles infection in hamster cochlea. *Acta Oto-Laryngologica* **(suppl) 514:** 111–116.
Gussen, R., Canalis, R. (1982) Muco-mycosis of the temporal bone. *Annals of Otology, Rhinology and Laryngology* **91:** 27–32.
Hanna, E., Hughes, G., Eliachar, I., Wanamaker, J., Tomford, W. (1993) Fungal osteomyelitis of the temporal bone: a review of reported cases. *Ear, Nose, and Throat Journal* **72:** 537–541.
Kim, M. H., Rodey, G. E., Good, R. A., Chilgren, R. A., Quie, P. G. (1969) Defective candidacidal capacity of polymorphonuclear leukocytes in chronic granulomatous disease of childhood. *Journal of Pediatrics* **75:** 300–303.
Lehrer, R. I., Cline, M. J. (1971) Leukocyte candidacidal activity and resistance to systemic candidiasis in patients with cancer. *Cancer* **27:** 1211–1217.
Louis, T., III, Lockey, M. (1974) Blastomycosis of the middle ear. *Southern Medical Journal* **67:** 1489–1491.
McGill, T. J. (1978) Mycotic infection of the temporal bone. *Archives of Otolaryngology – Head and Neck Surgery* **104:** 140–144.
Meyerhoff, W. L., Paparella, M. M., Oda, M., Shea, D. (1979) Mycotic infections of the inner ear. *Laryngoscope* **89:** 1725–1734.
Papadimitriou, J. M., Ashman, R. B. (1986) The pathogenesis of acute systemic candidiasis in a susceptible inbred mouse strain. *Journal of Pathology* **150:** 257–265.
Rarey, K. E., Davis, L. E. (1993) Temporal bone histopathology 14 years after cytomegalic inclusion disease: a case study. *Laryngoscope* **103:** 904–909.
Wade, J. C. (1993) Epidemiology of *Candida* infections. In *Candidiasis. Pathogenesis, Diagnosis, and Treatment.* 2nd Edition. (Bodey, G. P., ed.) Raven Press, New York, pp. 85–107.

Address for correspondence:
Dr R. B. Ashman,
Department of Pathology,
University of Western Australia,
Nedlands, W.A. 6009. Australia.

Fax: 61 9 346 2891