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The ultrastructure of cholesterol granuloma of the middle ear: an electron microscope study

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Cholesterol granuloma was first described by Manassé in 1917 but it has been ignored until Birrell (1956), Sheehy and McKibben (1956) and Friedmann (1959) drew renewed attention to this peculiar lesion, often confused with epidermoid cholesteatoma, even by experienced pathologists (Anderson, 1971).

Basically a histopathologic entity (Friedmann, 1959, 1974), it may be suspected at operations on the mastoid process or in cases presenting with features of so-called 'idiopathic haemotympanum' (Ranger, 1949; Sheehy and McKibben, 1956; Sheehy, Linthicum and Greenfield, 1969).

Cholesterol granuloma forms part of the inflammatory granulation tissue of otitis media, in the middle ear cleft, and it can be more accurately identified or confirmed only by the histological examination of a specimen removed at operation (Friedmann, 1959).

Cholesterol may be deposited in the infected, inflamed, or haemorrhagic middle ear tissues. The characteristic features are those of chronic inflammatory granulation tissue containing large numbers of rhomboid clefts of cholesterol crystals, dissolved during processing, surrounded by foreign body giant cells (Figs. 1 and 2). There is usually some clear evidence of recent and old haemorrhage, with haemoisiderin pigment.

The histopathological features differ fundamentally from those of epidermoid cholesteatoma although the conditions may occur together. In such cases, cholesterol crystals may be present 'in' a cholesteatoma, leading to erroneous conclusions about its pathogenesis.

The pathogenesis of cholesterol granuloma has aroused great interest. The ubiquitous nature of cholesterol, and in particular its presence in large quantities in the blood, points to the blood as its main source of origin. It may also be formed in mucus, pus or necrotic tissue (Siirala, 1964; Friedmann, 1974; and others).

Cholesterol resists absorption by giant cells. This paper presents some electron microscopic observations on clinically and histologically diagnosed cases of cholesterol granuloma, with particular reference to its ultrastructure and to the role of the giant cells.

From the Ear Research Institute, Los Angeles, California.

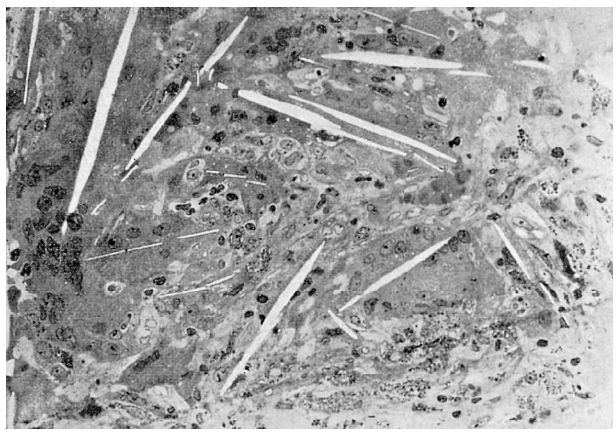

Fig. 1

Cholesterol granuloma: semi-thin section stained with Toluidin-blue. Multinucleated giant cells embedded in inflammatory granulation tissue contain large numbers of nuclei clustered in the centre of the cell or lined up along the outer cell membrane. There are slender cholesterol needles of various lengths in the cells, some short needles forming an interrupted line in the cytoplasm. (Case 4) ×225.

Case reports*

Case 1. Male age 45. This patient presented in 1977 with long-standing hearing loss and a history of intermittent bleeding from the ear, with pain. A biopsy of an aural polyp about nine years ago showed a cholesterol granuloma. On examination, there was a bulging granuloma from the left tympanic membrane, clinically diagnosed as a cholesterol granuloma. Radiography showed a partly pneumatized mastoid process with some sclerosis and features suggestive of cholesterol granuloma.

Treatment: Mastoidectomy was performed and cholesterol granuloma was found in all the peripheral cells, in the mastoid antrum and in the epitympanic recess.

Case 2. Male age 48. This patient was first seen in November 1973 with a hearing loss of 20 years' duration in the left ear. He had multiple infections of the ear as a child. On examination a blue-brown

*The patients were seen at the Otologic Medical Group Inc., Los Angeles, and operated on by Drs Sheehy and De La Cruz.

coloured left tympanic membrane was noted, with a conductive hearing loss of 68 db.

Clinical diagnosis: Left idiopathic hemotympanum. Myringotomy revealed some brown fluid characteristic of this condition. Radiography showed a pneumatized mastoid process with opacity and bone erosion.

At operation: There were big clumps of cholesterol granuloma in the peripheral mastoid cells and lateral to the ossicles in the epitympanum. The patient made an uneventful recovery and was well when last seen in March 1976.

Case 3. Female, age 30. This patient was treated in December 1971 for chronic otitis media and mastoiditis (left) by mastoidectomy. Cholesteatoma was found, with purulent exudate in the mastoid cells. Foul discharge has continued since, on and off, and she also received anti-TB treatment.

The patient was first seen at the Otologic Medical Group in September 1977. The diagnosis was that of left chronic otitis media, and a left revision tympanoplasty with mastoidectomy was performed.

A huge cholesterol granuloma was found in a previous modified radical mastoidectomy area filling the whole mastoid process and middle ear. This mass, of about 2 cm, occluded the external auditory meatus completely and the well-pneumatized temporal bone contained cholesterol granuloma in all cells. The mucosa appeared to be normal. No cholesteatoma was seen. Microscopy confirmed the cholesterol granuloma. No Acid-Alcohol-Fast Bacilli were seen or grown.

Case 4. Ten-year-old boy, seen in 1978, complaining of gradual hearing loss over three years accompanied by intermittent discharge from both ears. On examination both tympanic membranes were found to be perforated and there were conductive hearing losses of 40 and 50 db, in the right and left ears respectively. Tympanoplasty-and-mastoidectomy was performed on the left ear (the right to be operated subsequently). There was extensive cholesterol granuloma in the middle ear cleft, and the malleus and incus were missing.

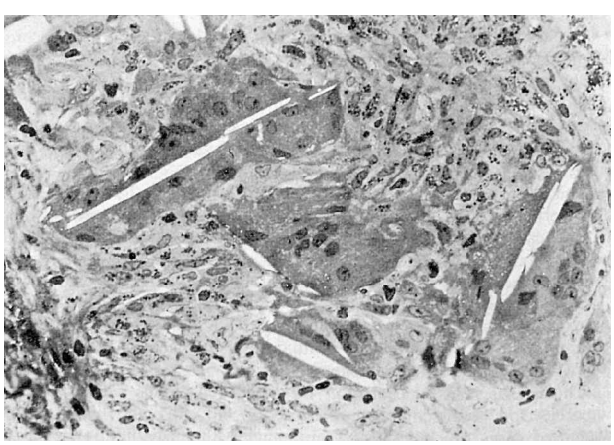

FIG. 2

A group of multinucleated giant cells with phagocytosed cholesterol needles. Semi-thin section stained with Toluidin blue. (Case 4) ×350.

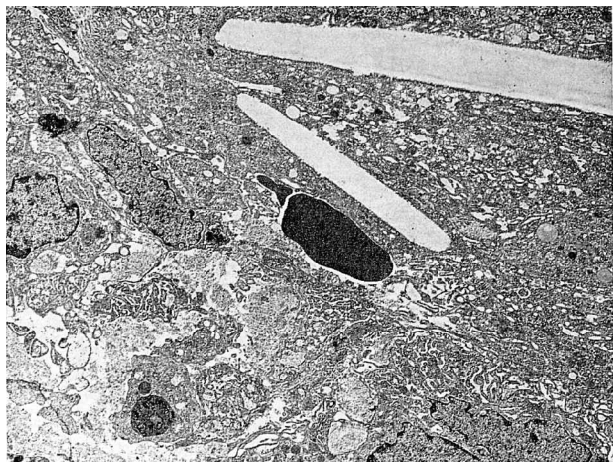

FIG. 3

Detail of giant cells containing two slender cholesterol needles and a red blood corpuscle. Note histiocytes in surrounding granulation tissue. (Case 1) ×3,800.

Case 5. Woman of 50, seen in 1978, complaining of hearing loss and smelly discharge from the left ear of about one year's duration. On examination the tympanic membrane was found to be perforated and there was some aural granulation tissue. Chronic otitis media was diagnosed and tympanoplasty with mastoidectomy performed. The middle ear cleft was filled with epidermoid cholesteatoma and cholesterol granuloma.

Case 6. A young man of 20 had been treated surgically (tympanoplasty and mastoidectomy) for bilateral chronic otitis media. Now complaining of plugging and discharge from the right ear.

On examination the right tympanic membrane was found to be perforated. The left ear showed the usual appearances after modified radical mastoidectomy.

Treatment: Simple mastoidectomy was performed on the right ear and large masses of cholesterol granuloma were removed for investigation under the electron microscope.

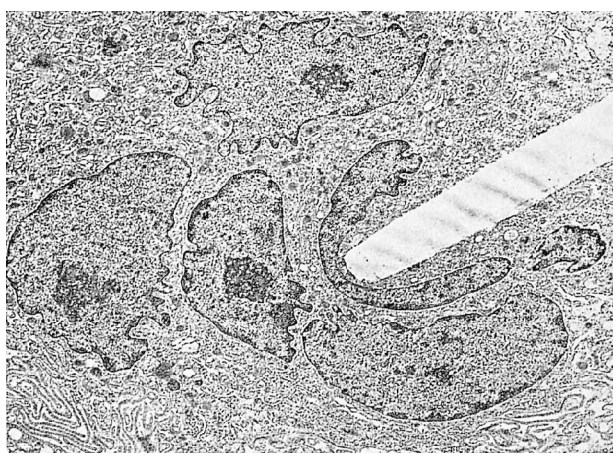

FIG. 4

Detail of multinucleated giant cell. An indented nucleus appears to be spiked by a cholesterol needle. The cytoplasm contains stacked cisternae of rough endoplasmic reticulum, mitochondria and lysosomes. (Case 1) ×11,000.

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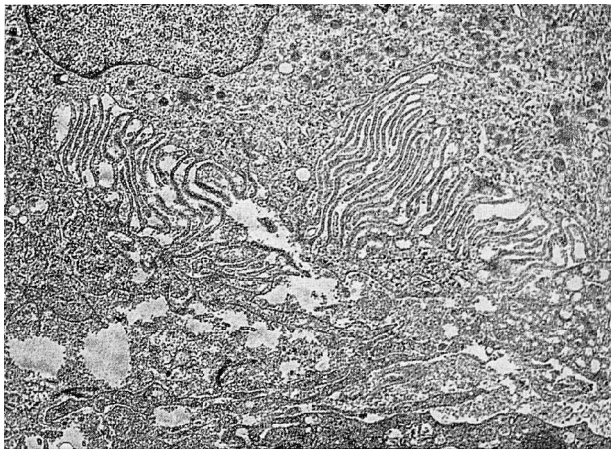

FIG. 5

Detail of giant cell showing stacked cisternae of rough endoplasmic reticulum and many lysosomes. (Case 1) ×22,000.

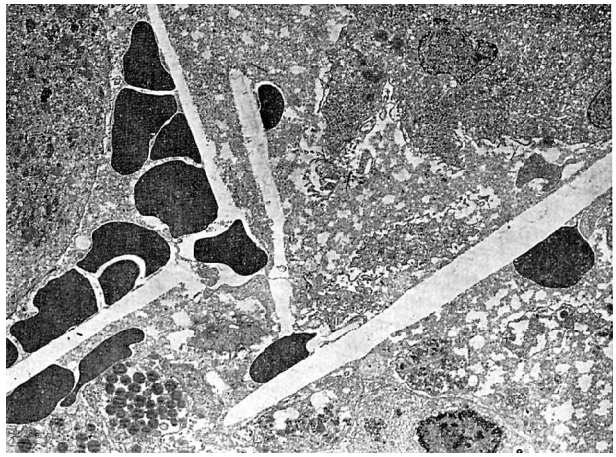

FIG. 7

There are cholesterol needles in a pool of red blood corpuscles. Parts of giant cells also seen containing large numbers of mitochondria. (Case 1) ×3,800.

Microscopical observations

Light microscopy

All examined specimens displayed the characteristic microscopical features of cholesterol granuloma; i.e. there were large numbers of cholesterol crystals of various sizes and shapes, some rhomboid, others slim, dart-or needle-shaped, surrounded by multinucleated giant cells embedded in inflammatory granulation tissue. There were polymorphonuclear leukocytes, active histiocytes and many plasma cells present. Many of the giant cells contained slim cholesterol needles, as clearly seen in semi-thin sections (Figs. 1 and 2).

Electron microscopy

The ultrastructure of the giant cells resembled that of other foreign-body type giant cells. The cytoplasm contained large numbers of mitochondria and lysosomes (Figs. 3, 4, 5 and 6) in the endoplasmic reticulum, composed of stacked cisternae of rough type studded with ribosomes (Figs. 4 and 5). The nuclei were grouped in the central part of the cell or aligned alongside the outer cell membrane. Large nucleoli were prominent in many nuclei (Fig. 4). The Golgi apparatus consisted of stacked vesicles and cisternae.

The significant distinction from other giant cells lay in the presence of the cholesterol needles included in them (Figs. 3 and 4); these appeared as empty spaces surrounded by mitochondria, and they were partly covered by some darkly stained lipid-like amorphous material. Some of the longer needles seemed to be piercing the cytoplasm or undermining it. Others appeared to be spiking an indented nucleus (Fig. 4).

Short or longer cholesterol needles were scattered in the granulation tissue, frequently in a pool of blood corpuscles, or surrounded by plasma cells of typical endoplasmic pattern (Figs. 3, 7 and 8).

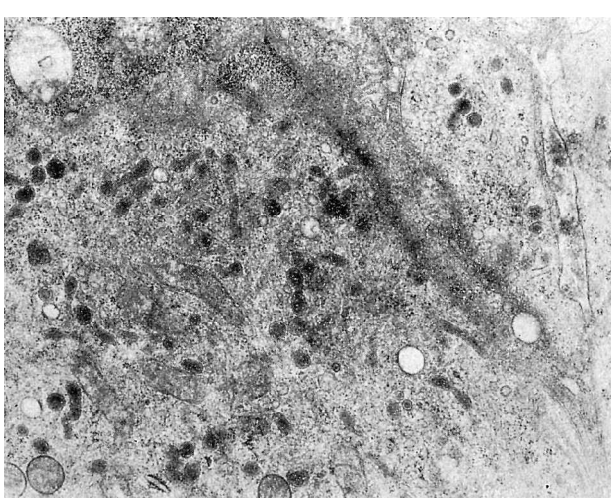

FIG. 6

Detail of cytoplasm of giant cell containing many membrane-bound lysosomes. (Case 4) ×33,000.

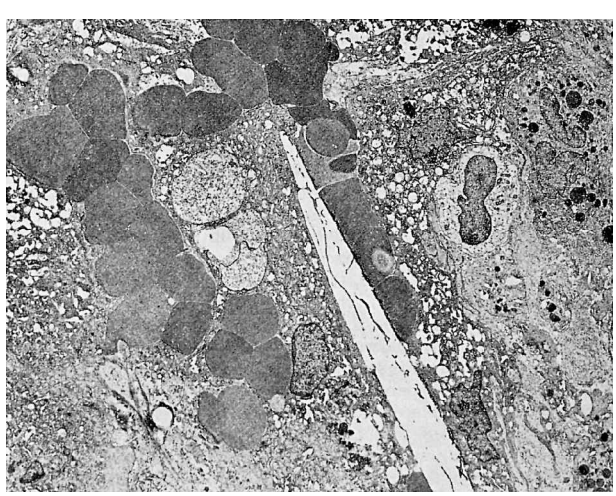

FIG. 8

Shows the tip of a dart-shaped cholesterol needle in a giant cell surrounded by many red blood corpuscles. Note polymorphonuclear granulocytes in the inflammatory granulation tissue. (Case 6) ×3,750.

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Discussion

Arnold (1971) has defined the cholesterol granuloma as a 'particular type of reaction of the chronically irritated middle ear mucosa'. Cholesterol is a substance of great resistance to the normal processes of absorption, and the deposited crystals tend to provoke an intensive granulomatous reaction in which foreign-body type giant cells play a leading role.

Under the light microscope the giant cells are seen to be isolating the deposited crystals from the surrounding inflammatory granulation tissue, although there is little or no evidence of their success in disposing of them. In the present study attention has been focused on the ultrastructure of the giant cells and their phagocytic property.

The giant cells of cholesterol granuloma contain, besides a large number of nuclei with large nucleoli, a rich endoplasmic reticulum with stacked cisternae of mainly rough type studded with ribosomes. There are vast numbers of mitochondria and parts of the cytoplasm are crowded with lysosomes. Giant cells with indented nuclei and rich in lysosomes resemble actively phagocytic histiocytes. Since the giant cells in cholesterol granuloma possess similar ultrastructural properties they seem to be well equipped for an active role in the absorption of cholesterol crystals formed in the affected tissues of the middle ear.

Giant cells develop in a toxic environment (Jones-Williams, Fry and James, 1972) and act as phagocytic cells. There is some evidence for a selective release of lysosomal enzymes from cell populations containing multinucleated giant cells (Papadimitrious and Wee, 1976) and similar mechanisms may be operating in a cholesterol granuloma.

Under the light microscope the giant cells are seen to be enveloping and isolating the larger crystals of cholesterol; in semi-thin sections the smaller needles of cholesterol can be recognized inside the giant cells: this feature has been largely ignored in earlier descriptions (Friedmann, 1959).

Such tiny inclusions can best be studied in thin sections under the transmission electron microscope. In the material studied, the giant cells frequently contained one, two or more rhomboid or short dart- or needle-shaped inclusions of cholesterol appearing as empty spaces ranging from about 0.5 to 2 μm in length. Well embedded in the cytoplasm, piercing or undermining it in some cells, the crystals were surrounded by mitochondria endosplasmic cisternae and scattered lysosomes. Near the tip of cholesterol needles, or when two of them appeared to be colliding in the cell, some of the organelles or collagen fibres were compressed.

As regards the source of the cholesterol deposits it is significant to find that many of the short or longer needles lying in the inflammatory granulation tissue of the cholesterol granuloma were surrounded not only by plasma cells, lymphocytes and histiocytes, but by many red blood corpuscles. Experimental evidence points to blood as the main source of cholesterol (Friedmann, 1959; Beaumont, 1966; and others). Sakamoto (1967), Lim and Birck (1971) and Arnold and Von Ilberg (1974) have noted the formation of cholesterol crystals from decomposed blood. Lim and Birck (1971) have noted that a cholesterol granuloma may develop from serous or catarrhal otitis media, as confirmed by one of the present writers (Friedmann, 1974). Idiopathic haemotympanum, a clinical rather than a pathological entity often associated with cholesterol granuloma, points in the same direction.

Sakamoto (1967) suggests that cholesterol may be formed in the giant cells, but this has not been observed in similar giant cells; for instance, in Wegener's granulomatosis (Friedmann, 1971) or in experimentally-produced foreign-body granuloma (Black and Epstein, 1974). The observations here presented have been interpreted as phagocytosis of the cholesterol crystals by active giant cells.

Summary

The ultrastructure of giant cells in cholesterol granuloma is described, with particular reference to their role in the absorption of cholesterol needles.

Cholesterol is a highly resistant substance in its crystalline form and it is confirmed that the giant cells are endowed with endoplasmic structures and organelles and are partly crowded with lysosomes, as evidence of the active role of the giant cells in the absorption and removal of cholesterol formed in the granuloma from blood, mucus or necrotic material.

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REFERENCES

Anderson, W. A. D. (1971) *Pathology,* VIth ed. St. Louis C. V. Mosby & Co., Vol. 2, p. 1063, Fig. 25.29.

Arnold, W. (1971) Reaktionsformen der Mittelohrscheimhaut. *Archiv. fur. Ohren-, Nasen und Kehlkopfheilkunde,* **216:** 440.

Arnold, W., and Ilberg, Ch.v. (1974) Ultrastrukturelle Veranderungen an der Mittelohrschleimhaut bei der idiopathischen hamorrhagisch-serosen Mittelohrentzundung (Otitis nigra). *Archiv. für Klinische und experimentelle Ohren-, Nasen- und. Kehkopfheilkunde,* **208:** 15.

Beaumont, G. D. (1966) The effect of exclusion of air from pneumatized bone. *Journal of Laryngology and Otology,* **80:** 236.

Birrell, J. F. (1956) Black cellular cholesteatosis in children. *Journal of Laryngology and Otology,* **70:** 260.

Black, M. M. and Epstein, W. L. (1974) Formation of multinucleate giatn cells in organized epithelioid cell granulomas. *American Journal of Pathology,* **74:** 263.

Friedmann, I. (1959) Epidermoid cholesteatoma and cholesterol granuloma: Experimental and human. *Annals of Otology, Rhinology and Laryngscope,* **68:** 57.

Friedmann, I. (1971) The changing pattern of granulomas of the upper respiratory tract. *Journal of Laryngology and Otology,* **85:** 631.

Friedmann, I. (1974) *Pathology of the Ear.* Oxford, Blackwell Scientific Publications.

JONES-WILLIAMS, W., FRY, E. and JAMES, E. M. (1972) The fine structure of beryllium granulomas. *Acta Pathologica, Microbiologica Scandinavica (A) (Suppl),* **233:** 195.

LIM, D. and BIRCK, M. (1971) Ultrastructural pathology of the middle ear mucosa in serous otitis. media. *Annals of Otology, Rhinology and Laryngology,* **80:** 838.

MANASSE, P. In Ohrenheilk, Gegenw. (1917) *Handbuch der Pathologischen Anatomie des Mensclichen Olvres, Wiesbaden,* Vol. 9, p. 51.

PAPADIMITRIOUS, J. M. and WEE, S. H. (1976) Selective release of lysosomal enzymes from cell populations containing multinucleated giant cells. *Journal of Pathology,* **120:** 193.

RANGER, D. (1949) Idiopathic haemotympanum. *Journal of Laryngology and Otology,* **63:** 672.

SAKAMOTO, T. (1967) E. M. Studies of the origin of cholesterin crystals in cholesterol granuloma. *Journal of Otorhinolaryngological Society of Japan,* **70:** 1926.

SHEEHY, J. L. and MCKIBBEN, B. G. (1956) Idiopathic hemotympanum. Review of the literature and report of three cases. *Laryngoscope,* **66:** 1291.

SHEEHY, J. L., LINTHICUM, F. H. and GREENFIELD, E. C. (1969) Chronic serous mastoiditis, idiopathic hemotympanum and cholesterol granuloma of the mastoid. *Laryngoscope,* **70,** 1189.

SURALA, U. (1964) Otitis media adhaesiva. *Archives of Otolaryngology,* **80:** 287.