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Tonsillomycosis

A myco-histopathological study

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Introduction

STIMULATED by the considerable interest in the relation of commonly occurring moulds to allergic respiratory diseases in the last twenty years, Bernard *et al.* (1962) studied fungi observed in 100 routine consecutive tonsillectomies. They obtained positive cultures in 49 cases, mainly *Aspergillus* and *Candida* species and, less commonly, *Alternaria, Penicillium, Hormodendrum,* etc. amounting to 15 species in all. Pathological studies revealed visible colonies in the crypts of 18 cases accompanied by a hyperplasia of the epithelium of the crypts, without any granulomatous reaction. They concluded that these fungi in the tonsils might act as antigens that induce respiratory allergies.

This view was supported by Jokinen *et al.* (1976), who advised tonsillectomy and adenoidectomy in some cases for that reason. They carried out a similar study on 147 tonsillectomy and 134 adenoidectomy cases, compared to a control group of 68 cases, with results very similar to those of Bernard *et al.* (1962).

Positive cultures were obtained in 64 per cent of tonsils, including 41.4 per cent *Candida albicans*, 8 per cent for each of *Saccharomyces* and *Aspergillus* species, with other species less common. Twenty-one per cent of adenoids gave positive cultures. In the control group, the same species were identified in a smaller percentage of cases (69 per cent) with a slightly greater prevalence of *Candida* (51.5 per cent) and fewer *Aspergilli* (1.5 per cent). Histologically, colonies were visible in the crypts of 23 per cent of the tonsillectomy cases and 5 per cent of adenoid tissue, but without evidence of significant reaction. These organisms were similar to the well-known oral flora of normal individuals.

Rüge (1885) found granules of *Actinomyces* in tonsillar crypts. Lord (1910) and Emmons (1938) demonstrated *Actinomyces* by direct examination, in 37 per cent of 100 pairs of extirpated tonsils and isolated it by

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culture in 14 per cent. Wilkinson (1929) examined tissue from 10,000 tonsillectomy cases; by histological examination he found granules resembling *Actinomyces* in 1.8 per cent. Grüner (1969) reported 16.5 per cent *Actinomyces* (17 positive cases) in 103 tonsillectomy cases. In none of his positive cases, however, were the colonies recognized at the first examination. He found that PAS (Periodic Acid-Schiff) stain was excellent for screening purposes and stated that it was customary to regard *Actinomyces* granules in the tonsils as mere saprophytes.

In 1971, Grüner reported a histological survey of autopsy material for the prevalence of *Actinomyces* in normal tonsils. He found *Actinomyces* colonies in the tonsils of 9 of 22 bodies and suggested that tonsillar crypts were a normal habitat of such colonies. Peabody and Seabury (1960) considered *Actinomyces* an opportunistic microbe, especially in individuals with compromized host defences.

A number of studies have reported culture of several mycotic organisms from oropharyngeal tissues. Since some of these organisms can exist as saprophytes in the human mouth and pharynx, their role in the production of disease is uncertain. This study was undertaken to correlate the culture of fungus organisms with histopathological findings in cases of chronic inflammation of the palatine tonsils and adenoids.

Materials and Methods

Two-hundred-and-twenty-seven consecutive subjects with chronic tonsillitis were examined in the E.N.T. outpatient clinic of Fakhry Hospital, in the Eastern District of Saudi Arabia. These cases represented a broad age range of male and female patients—mainly labourers—of several nationalities.

A culture specimen for mycological examination was obtained by bisecting the removed tonsils under strictly aseptic conditions. A cross-section slice of tonsillar tissue, approximately 5 mm. thick, was obtained from the centre of each of the halves. This was macerated and processed for culture. Debris from tonsillar crypts was easily expressed in 19 of the cases. This material was also examined by cultural and histopathological means but was not included separately in the case count. Brain-heart infusion agar (B.H.I.) and Sabouraud's agar media were used with and without chloramphenicol (0.05 mg/ml).

For *Actinomyces*, thioglycolate broth was used at 37°C. After colonies had developed, those suspected of being *Actinomyces* spp. were picked and streaked on BHI plates. Duplicate plates for each medium were used for each sample. Plates for the growth of yeasts and *Actinomyces* were incubated at 37°C and the others at room temperature for moulds. Plates were examined twice weekly and only those with no growth for one month were considered negative. Identification of isolated yeasts was based on both morphological and biochemical methods (Ajello *et al.* 1966).

Mould isolates were identified morphologically as a prelude to their

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possible detection histopathologically (Gilman, 1959; Barnett, 1960; Booth, 1971; Barnett and Hunter, 1972). Identification of isolated Chloramphenicol-resistant bacteria was irrelevant in our study and was not performed.

The remaining portions of the bisected tonsils were fixed in 4 per cent neutral buffered formalin solution, dehydrated, embedded and cut at 5 microns thick by routine methods. The sections were stained with Hematoxylin and Eosin (H & E), Periodic Acid-Schiff (P.A.S.), Brown-Bren Gram stain (B & B) and Gomori's Methenamine silver stain (G.M.S.) (Luna, 1968) for histopathological examination.

Two patients presented with sore throat several weeks after tonsillectomy. Foreign material adherent to the inflamed surgical site was removed for culture and histopathological examination.

Results

In the 227 cases examined by culture, *Actinomyces* was isolated in 69 (30.40 per cent), yeasts in 21 (9.25 per cent), moulds in 21 (9.25 per cent), mixed isolates in 7 (3.08 per cent) and chloramphenicol-resistant bacteria in 56 cases (23.35 per cent) (Table I).

In addition, 17 of the 19 specimens of the debris expressed from tonsillar crypts (Fig. 1) showed the same cultural and histopathological results as the tonsillar tissue from the same cases (Fig. 2).

In H&E stained sections, colonies of *Actinomyces* were identified in 69 cases as round-to-irregularly oval structures (the 'sulphur granules'), dark blue to purple. In this study the colonies were invariably external to the tonsillar epithelium, most often within the crypt lumina. There was very little cellular response to the organisms, usually a small number of mononuclear cells only, and scant proteinaceous debris (Figs. 3 and 4). The adjacent epithelium was normal except in several cases in which it was slightly hyperplastic and infiltrated by small numbers of neutrophils. We were unable to demonstrate other organisms in these cases.

With GMS stain, individual coccoid and filamentous forms were clearly evident (Fig. 5). The colonies were variably PAS-positive but morphology of the organism was indistinct. With B&B stain, the organisms appeared slightly larger than with PAS and GMS. Coccoid forms tended to be blue and filaments pink with B&B gram stain but this was not constant; in some sections the opposite prevailed or combinations occurred.

The two cases of post-tonsillectomy pharyngitis had similar appearances. Yellowish debris was adherent to the inflamed pharyngeal mucosa (Fig. 6). Examination revealed retained silk suture material and *Actinomyces* colonies (Fig. 7).

Bacterial colonies, mostly micrococcal chains, were observed in only 25 cases (11.01 per cent). In the remaining cases with positive cultures (bacteria, yeasts and moulds), no micro-organisms were seen histopathologically.

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TABLE
RESULTS OF CULTURE OF 227 CASES

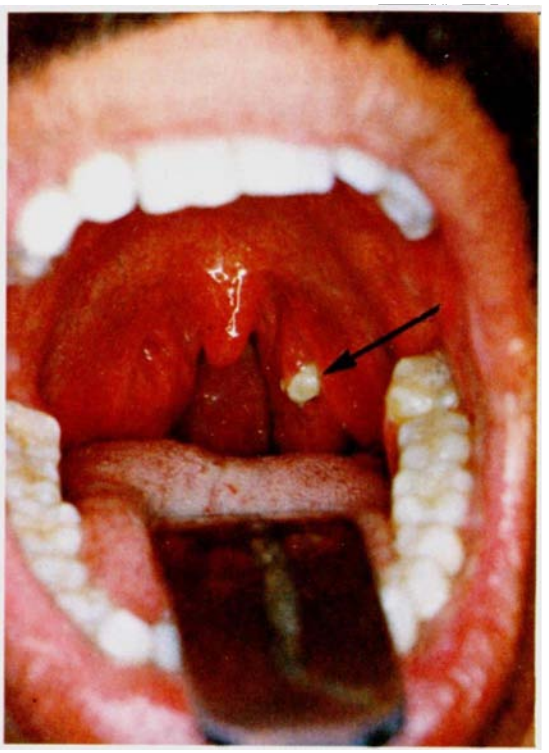
Organism		Number of isolates	Percentage
Actinomyces spp.	69		30.40
Moulds:	21		9.25
Aspergillus flavus		7	
Aspergillus niger		4	
Aspergillus terreus		1	
Penicillium variabile		3	
Penicillium lanosum		1	
Penicillium chrysogenum		1	
Penicillium citrinum		1	
Spicaria simplicissima		1	
Fusarium poae		1	
Mucor lausannensis		1	
Yeasts:	21		9.25
Candida albicans		12	
Saccharomyces spp.		8	
Rhodotortula sp.		1	
Mixed isolates:	7		3.08
Aspergillus niger + *Candida albicans*		2	
Bacteria + *Candida albicans*		2	
Aspergillus niger + *Saccharomyces* sp.		1	
Bacteria + *Saccharomyces* sp.		1	
Aspergillus niger + bacteria		1	
Bacteria	56		24.67
No growth	53		23.35
Total	227		100.00

Discussion

Presumptive identification of classes of micro-organisms in tissue is possible with good stains. For fungi, GMS or Gridley fungus stains delineate the morphology of the organism, and most are PAS-positive. Bacteria can be stained well with preparations for that purpose. *Actinomyces* share morphologic characteristics with true fungi (hyphae) but the small size is a definitive feature and its reaction to Gram stains distinguishes it from true fungi. That the *Actinomyces* in these tissues is saprophytic and not pathogenic is indicated by the absence of response to the colonies. When lesions are produced by this organism there are characteristically purulent granulomas, with 'sulphur granules' embedded in purulent exudate. No such reaction was seen.

The isolation of *Actinomyces* in culture and its demonstration histopathologically in some of our cases (30.40 per cent) is in accordance with

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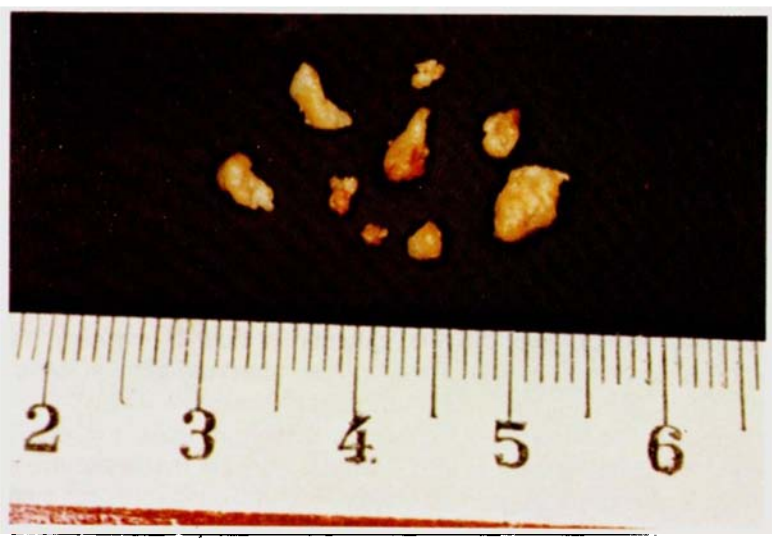


FIG. 1

(a) Debris in the tonsillar crypt (arrow) responsible for foreign body sensation.
(b) Debris expressed from crypt of tonsil shown in Fig. 1a.

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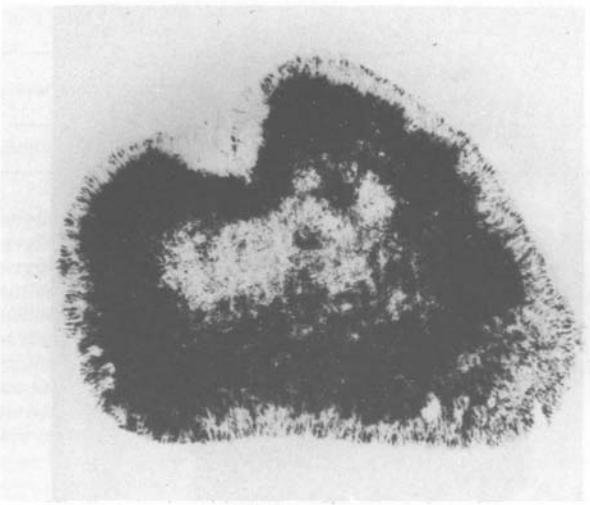


FIG. 2

Histological appearance of a single granule (ray fungus) in the debris expressed from tonsillar crypt (GMS stain X150).

the results of Rüge (1885), Lord (1910), Emmons (1938), Wilkinson (1929), Grüner (1969, 1971) and Peabody and Seabury (1960), but with different prevalences.

In 1969 Grüner postulated that the cause of the difference in prevalence of *Actinomyces* in his work, 16.5 per cent as compared to 1.8 per cent only in Wilkinson's series, may result from stricter criteria for tonsillectomy in recent years; thus, if *Actinomyces* colonies were a sign of tonsillar disease, a greater proportion would be expected in which fewer normal tonsils were removed (Grüner, 1971).

In our series, the vast majority of cases were adults who had been subjected to repeated treatment with antibiotics for recurrent sore throats. This may have been a factor in the difference in prevalence of isolated organisms in our series and those of previous workers.

The growth of *Actinomyces* colonies around retained sutures in the tonsillectomy wound site of two patients is further evidence that the organism is a common inhabitant of the pharynx and, at best, is an opportunist in its association with pharyngeal disease.

In spite of their isolation in culture, neither moulds nor yeasts were demonstrated histologically in our cases. It may be that these are normal commensals elsewhere in the oral cavity but not in the tonsillar crypts. On the other hand, the reported demonstration in tonsils of *Aspergilli* and other moulds by Bernard *et al.* (1962) and by Jokinen *et al.* (1976) are not convincing when based on their photographic illustrations. In our opinion,

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the morphology of the organisms which they illustrated in the tonsillar crypts are more suggestive of the 'ray fungus' of *Actinomyces* granules.

Finally we would like to mention that most of the cases with positive cultures and histopathological evidence of *Actinomyces* complained of

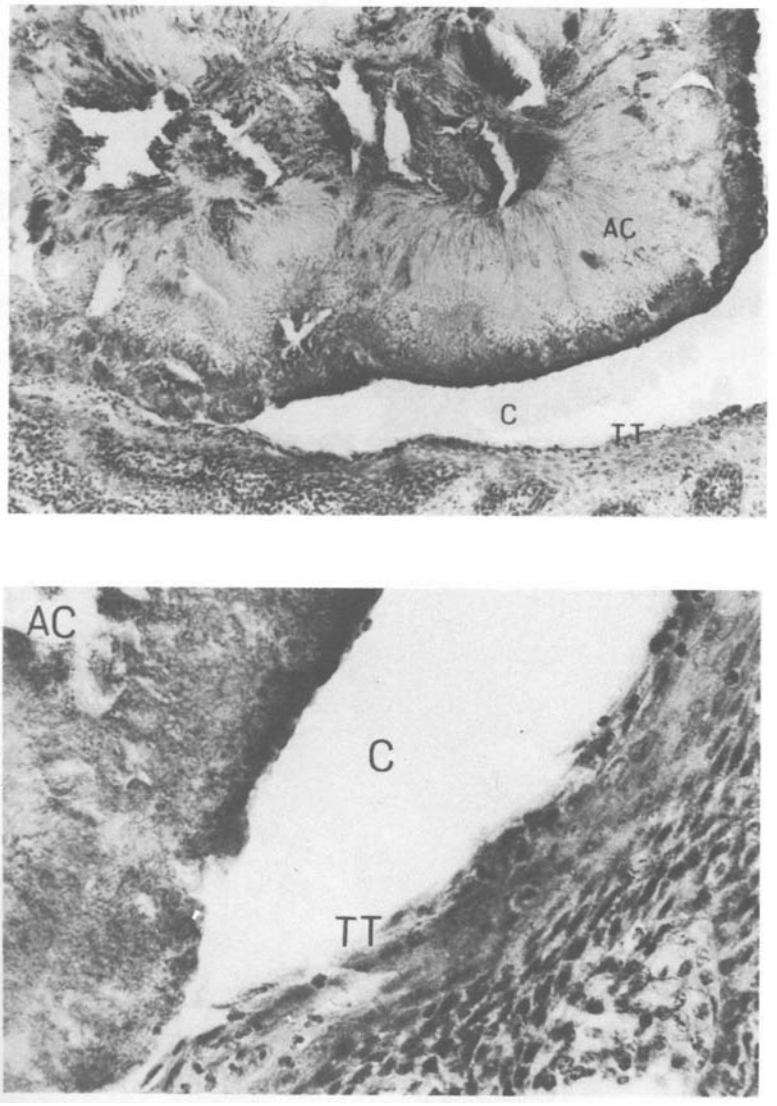


FIG. 3

Typical appearance of *Actinomyces* colony (AC) external to tonsillar tissue (TT). There is no inflammatory reaction. (Crypt space = c). (H & E stain) (a X90 & b X270).

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similar symptoms, viz. foetor oris, a sensation of foreign body in the throat, a dull ache of the tonsils immediately relieved by the expulsion of the debris from the crypts, and a constant fear of the yellowish spots that persisted in the tonsils even in quiescent periods. Species differentiation of the

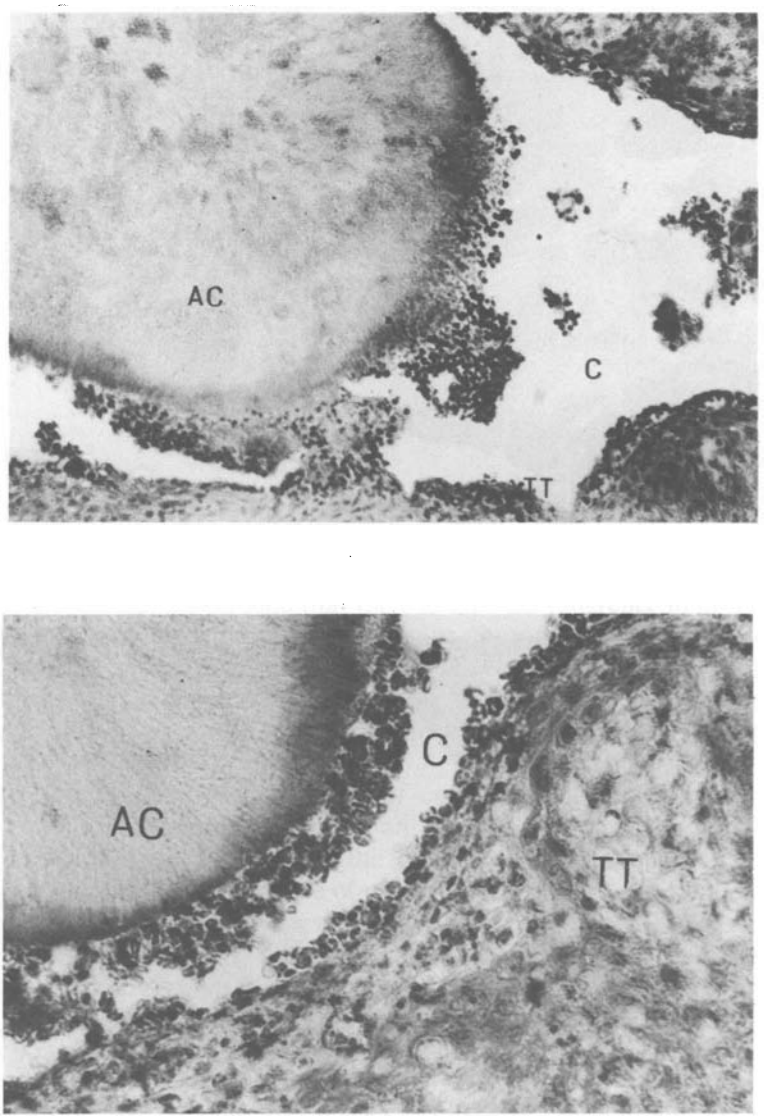
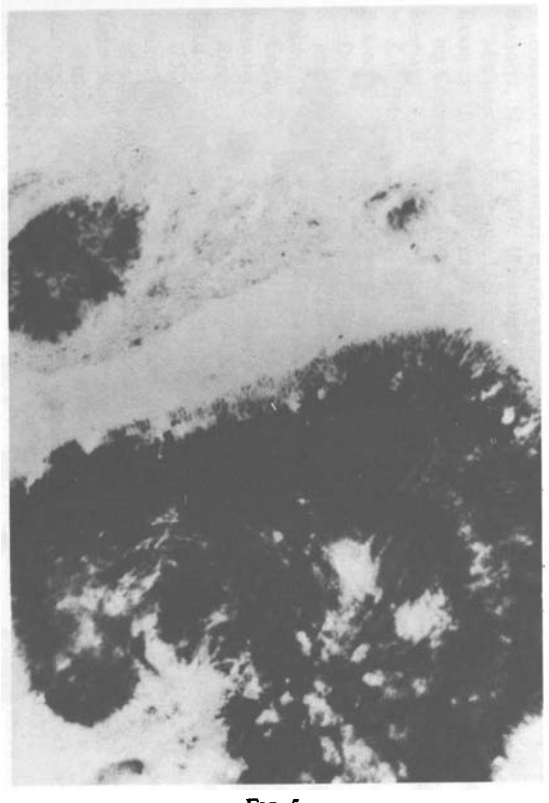


FIG. 4

Superficial inflammatory response present in several cases only. Scant infiltrate of neutrophils, lymphocytes and plasma cells with minimal focal hyperplasia of adjacent crypt epithelium. (AC = *Actinomyces* colony, TT = Tonsillar tissue, C = Crypt) (H & E stain) (a X90 & bX270).

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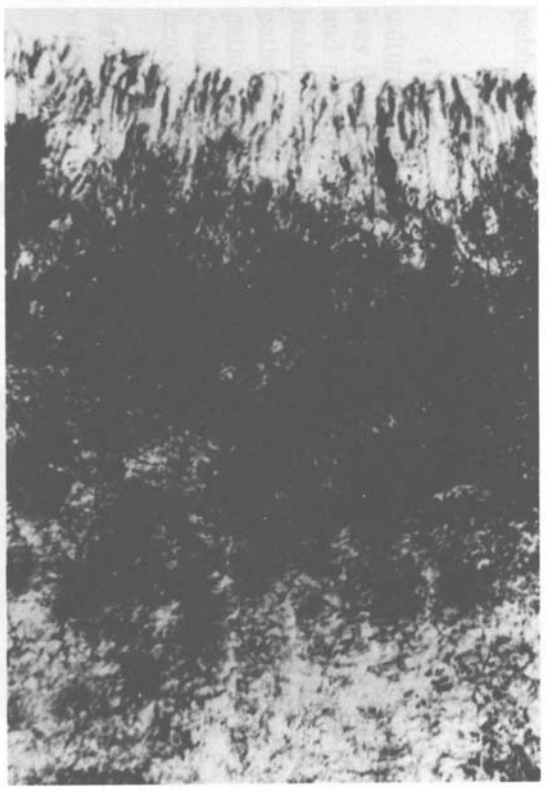


FIG. 5

(a) Tonsillar crypt containing actinomycotic granule (GMS stain X150).

(b) Periphery of actinomycotic granule, with filaments clearly outlined (GMS stain a X90 & b X270).

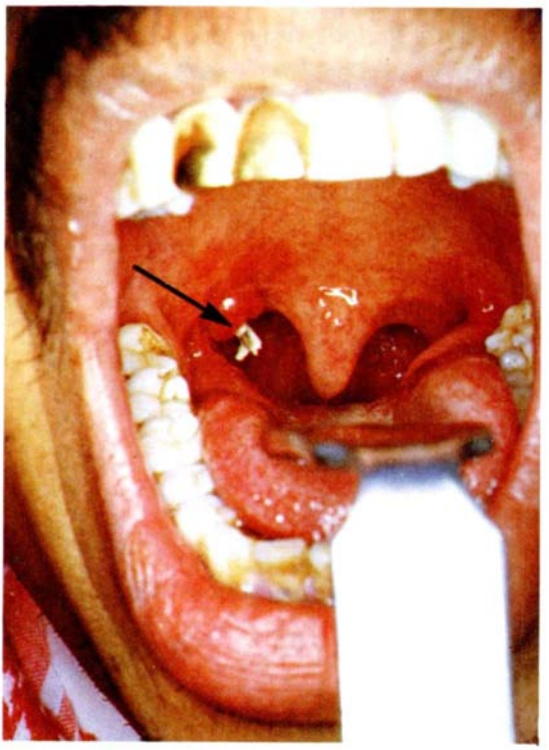


FIG. 6

Appearance of tonsillectomy site six weeks after surgery. Note inflammation and debris at location of suture (arrow).



FIG. 7

Actinomyces colonies ('sulfur granules') around silk suture material in tonsillectomy site, six weeks after surgery (X90).

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Actinomyces isolates in the present study and their inter-relation with *Actinomyces* spp. from other oral lesions will be discussed in a subsequent publication.

Summary

Tissues from 227 tonsillectomies, mainly in adults, were examined culturally and histopathologically for the presence of fungus. *Actinomyces* was demonstrated in 69 cases (30.40 per cent) by both methods. Yeasts and moulds were each isolated from 21 cases (9.25 per cent) and mixed isolates were obtained from 7 cases (3.08 per cent). There was no histopathological evidence of these organisms. Negative cultural and histopathological results were obtained in 53 cases (23.35 per cent). Chloramphenicol-resistant bacteria were cultured in 56 cases (25.11 per cent) but were seen histopathologically in only 25 cases (11.01 per cent).

In all cases in which *Actinomyces* or bacteria were present histopathologically, a minimal tissue response indicated that these organisms were saprophytes and not primary pathogens.

Acknowledgement

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