ACTINOMYCES ISRAELII, A CAUSE OF LACRIMAL CANALICULITIS IN MAN

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In systemic actinomycosis, Actinomyces israelii may affect the eye by extension through the orbit (O’Brien and Leinfelder, 1933) and occasionally the salivary and lacrimal glands may be invaded by direct extension (Cope, 1938). However, the fact that A. israelii can cause conjunctivitis or lacrimal canalicularis with no generalized systemic invasion has been recognized by only a few investigators. This fact is emphasized when one sees no references to this primary localized infection in current medical bacteriology or mycology texts, manuals, and reviews (Wilson, and Miles, 1957; Dubos, 1958; Lewis et al., 1958; Conant et al., 1954; Rosebury, 1944). Although Birge (1952) discussed the occurrence of Actinomyces bovis in lacrimal concretions, the confusion of nomenclature and the paucity of information characterizing isolates in ophthalmological literature do not permit one to know with certainty the etiologic agents involved.

Awerbach (1903), Gütler (1933), Ruys (1935), Hagedoorn (1940), Moore (1952), and Smith (1953) have satisfactorily identified Actinomyces species in concretions from the lacrimal canalculus of the eye. In some cases these reports, all from European workers, do not contain as complete a description of the strains as would be desired in light of our present knowledge of the genus. Recently strains of Actinomyces have been isolated from two cases of lacrimal canalicularis. It is the purpose of this report to present a complete description of these cultures which identifies them as A. israelii. A clinical report of these cases is to be published elsewhere.

MATERIALS AND METHODS

Strain 699 was isolated from small concretions obtained from the lacrimal duct by Dr. S. S.

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Roberts, Jr., Department of Ophthalmology, Duke University Medical Center. The granular material was washed in sterile saline, crushed, and portions inoculated into stab tubes of casitone glucose agar (Pine and Howell, 1956). Strain OS 131 was isolated from a mixed culture in thiglycolate broth inoculated with pus from a case of conjunctivitis by Dr. L. Turner, Department of Ophthalmology, McPherson Hospital, Durham, North Carolina. A discrete colony showing actinomyces-like growth was taken from the thiglycolate culture and was used to inoculate a stab tube of casitone glucose agar.

Inoculum from each of the above tubes was streaked on casitone glucose agar plates which were incubated at 37 C in a 95 per cent nitrogen-5 per cent carbon dioxide atmosphere. Single microcolonies of each strain, such as those shown in figure 2A and B, were picked and transferred to casitone glucose broth medium. The pure cultures obtained were then restreaked a second time. Microcolonies were picked and transferred to casitone glucose broth. The resultant cultures were used to inoculate casitone glucose agar stab tubes for use as stock cultures. These pure cultures were studied by the methods previously described by Pine and Howell (1956).

The relative growth under anaerobic and aerobic conditions was determined by inoculating casitone glucose agar slants with fixed amounts of inoculum and incubating with either a pyrogallol-sodium carbonate seal or a sodium carbonate-KH₂PO₄ seal. After 4 days of incubation the cells were washed off the slants, suspended in water to make a 10-ml suspension, and the optical density was read in a spectronic 20 colorimeter set at a wave length of 660 μ. The amounts of growth on various sugars were recorded as subjective values made by direct observation, or the amounts of growth were determined by optical density measurements and recorded as growth units according to the formula G.U. = OD ×
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Figure 1. Fragmentation of Actinomyces israelii, strains 699 and OS 131, into diphtheroid elements. 
A. Strain 699 mycelial granule, 48-hr culture from casitone broth with 0.05 per cent agar (900X). B. Strain OS 131 grown 48 hr on casitone agar, colonies under cover slip (350X). (Note: Figures reduced 27 per cent for reproduction here.)

1/dilution. Corrections were made for growth in control tubes to which no sugar was added.

Formic, acetic, and propionic acids were identified by collecting the volatile acids of 1 per cent glucose broth cultures by steam distillation, converting them to the hydroxamic acids according to the procedure of Wingerd (unpublished data, 1952) as given in Block et al. (1958). The acids were chromatographed on paper using the top layers of an amyl alcohol-formic acid-water mixture (75:25:75). Formic acid was determined quantitatively by the difference in total volatile acid before and after HgSO₄ oxidation. Acetic acid and propionic acids were determined quantitatively by simultaneous equations based on their respective Duclaux constants after HgSO₄ oxidation of the formic acid.

All cultures were incubated at 37 C. Unless otherwise stated, plate cultures were maintained in an atmosphere of 95 per cent nitrogen-5 per cent carbon dioxide, and tube cultures were maintained under a pyrogallol-sodium carbonate seal.

Both strains were tested for pathogenicity in hamsters (Hazen and Little, 1958). Four- to six-week-old hamsters were inoculated intraperitoneally and intradermally with strains 699 and OS 131 and with two strains of A. israelii isolated from cases of human actinomycosis. Cultures grown in 5 ml of casitone glucose broth for 48 to 72 hr were centrifuged, and the sediment re-suspended in 5 ml of sterile distilled water. The suspensions were homogenized and each animal was inoculated intraperitoneally with 0.5 ml and intradermally with 0.2 ml.

RESULTS

Depending on the medium used, strains 699 and OS 131 appeared as long filaments which fragmented into diphtheroid elements (figure 1A and B); branching filaments (figure 2A and B); or as coccobacillary and diphtheroid forms (figure 3A and B). Both strains were nonmotile, gram-positive, and not acid-fast. Isolate OS 131, when grown in thioglycolate broth (Difco) without a pyrogallol-sodium carbonate seal, formed typical bread crumb colonies 1 cm below the surface of the liquid to the bottom of the tube. The broth remained clear. However, isolate 699 formed granular growth 1 cm below the surface.
Figure 2. Colony morphology of two strains of *Actinomyces israelii* isolated from the eye. A. Strain 699 grown 24 hr on casitone agar. Colonies are under cover slip (360X). B. Strain OS 131 grown 48 hr on casitone agar. Colonies are under cover slip (360X). C. Strain 699 grown 3 days on casitone agar (45X). D. Strain OS 131 grown 5 days on casitone agar (45X). (Note: Figures reduced by ¼ for reproduction here.)
Figure 3. Microscopic morphology of Actinomyces israelii strains isolated from the eye. A. Strain 699, 48-hr casitone agar culture (1800X). B. Strain OS 131, 48-hr casitone agar culture (1800X). C. Strain 699 in pus from hamster (900X). D. Strain OS 131 in pus from hamster (900X). All slides were stained by the Gram stain. (Note: Figures reduced 35 per cent for reproduction here.)
from which comet-like streamers of diphtheroid-like cells of Actinomyces descended toward the bottom; the broth became cloudy within several days. Similar results were obtained in casitone glucose broth containing 0.05 per cent agar.

When streaked on agar plates of the casitone glucose agar or brain heart infusion agar (Difco), both strains formed the typical branching mycelium characteristic of the genus *Actinomyces* (figure 2A and B). After 6 to 8 days, the colonies of both isolates became smooth, round, and convex (figure 2C and D). The "molar tooth" type of colony, generally associated with *A. israelii*, was not observed. Colonies of strain 699 exhibited a gray-orange tint after 5 to 6 days of growth on the casitone glucose agar, whereas colonies of strain OS 131 remained white.

The results of fermentation experiments are summarized in table 1. In other experiments, growth determinations were made daily beyond the period of maximal growth. The following results were obtained. Strain 699 reached a maximal growth in 50 hr on glucose, fructose, maltose, sucrose, lactose, and mannitol. Mannose and xylose supported a limited amount of growth, whereas growth on rhamnose and salicin was negligible. Strain OS 131 reached maximal growth in 100 hr on glucose, fructose, mannose, maltose, sucrose, lactose, mannitol, and salicin. Mannose and rhamnose supported a limited amount of growth. The greatest difference between the fermentative abilities of the two strains was shown by their relative activity on salicin. Salicin supported the maximal rate of growth of strain OS 131 whereas strain 699 did not grow on this glucoside.

The results of physiological experiments are given in table 2. Strain 699 formed propionic acid, whereas strain OS 131 formed no chromatographically detectable amounts of this acid. In two fermentations, strain 699 formed 3.1 and 3.8 moles of formic acid, 3.5 and 4.0 moles of acetic acid, and 18.8 and 20.0 moles of propionic acid per ml of 1 per cent glucose casitone broth. In one fermentation, strain OS 131 formed no propionic acid, and 4.4 and 4.8 moles of formic and acetic acids, respectively.

Typical actinomycosis with granule formation was not obtained in the hamsters inoculated with strains 699 and OS 131. However, encapsulated abscesses, which ruptured on the 7th day, formed in the skin as a result of the intradermal injec-

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### Table 1

*Growth of Actinomyces israelii strains OS 131 and 699 on various substrates*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Strain OS 131</th>
<th>Strain 699</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid</td>
<td>Gas</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Cultures were grown 6 days in stagnant culture at 37°C, 0.06 per cent bromcresol purple was used to determine acidity. No obvious color change could be seen with the indicator between pH 6.5 and 6.1. Initial pH of the medium was 6.5.

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### Table 2

*Physiological reactions of Actinomyces israelii strains OS 131 and 699*

<table>
<thead>
<tr>
<th>Test</th>
<th>Strain OS 131</th>
<th>Strain 699</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gelatin*</td>
<td>No liquefaction</td>
<td>No liquefaction</td>
</tr>
<tr>
<td>Litmus milk*</td>
<td>Acid, soft curd with reduction</td>
<td>Acid, soft curd with reduction</td>
</tr>
<tr>
<td>Indole</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H₂S formation</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Relative anaerobic growth over aerobic growth†</td>
<td>2.85/0.08</td>
<td>5.40/1.4</td>
</tr>
<tr>
<td>Acids formed on 1% glucose broth:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acetic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Propionic</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Lactic</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Cultures incubated 6 days. All other cultures incubated 4 days prior to testing.
† Fractions recorded in growth units.
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tions. In figure 3 are shown thread-like branching hyphae observed in Gram stained smears of the pus taken from each animal. No abscesses were formed within 1 month as a result of intraperitoneal inoculations. In similar experiments with the two known strains of \textit{A. israelii}, one hamster had an encapsulated intraperitoneal abscess which contained typical sulfur granules with clubs. The second animal gave results identical with those of strains 699 and OS 131.

**DISCUSSION**

Strains 699 and OS 131 were catalase negative; they did not liquify gelatin, hemolyze whole blood, or digest casein; they grew better anaerobically than they did aerobically. These physiological characteristics are those most consistently reported for \textit{Actinomyces} species (Rosebury et al., 1944; Erikson, 1940; Suter, 1956; Pine and Howell, 1956; Hazen and Little, 1958). The remaining characteristics reported for this genus, i.e., H$_2$S formation, indole formation, and nitrate reduction, have been found to vary in the reports of different workers (Pine and Howell, 1956; Negroni and Bonfiglioli, 1939).

The sugar fermentations with strains 699 and OS 131 were in general agreement with the results reported previously in which better growth was obtained with most strains of \textit{Actinomyces} on glucose, maltose, and lactose (Pine and Howell, 1956). At present, however, sugar fermentations should serve only to characterize a given strain rather than distinguish species of \textit{Actinomyces}. For example, in Bergey’s Manual of Determinative Bacteriology, 7th Edition (Breed et al., 1957) it will be seen that mannitol is fermented by \textit{A. israelii} but not by \textit{A. bovis}. However, this sugar did not support significant growth of one strain of \textit{A. israelii} isolated from a case of human cervico-facial actinomycosis (Pine and Howell, 1956) and was found to give variable results with strains of \textit{Actinomyces} isolated from the human mouth (Ludwig and Sullivan, 1952). Negroni (1938) reported that \textit{Actinomyces discofiliatus} from osteomyelitis produced no acid from mannitol, and Negroni and Bonfiglioli (1939) reported strains of \textit{A. israelii} produced little or no acid from mannitol.

Sugar fermentations do serve to distinguish \textit{Actinomyces} strains from organisms which form acid and gas since, to date, no \textit{Actinomyces} has been reported to form gas from carbohydrates.

In this respect, the results obtained with strains 699 and OS 131 are in agreement with the results of other investigators (Rosebury et al., 1944; Erikson, 1940; Negroni and Bonfiglioli, 1939).

Several strains of \textit{Actinomyces} studied previously fermented glucose with the formation of lactic, succinic, formic, and acetic acids (Pine and Howell, 1956). In the present study, strain OS 131 also formed lactic, formic, and acetic acids; succinic acid was not determined. However, strain 699 formed not only lactic, formic, acetic, but also propionic acid; succinic acid was not determined. The significance of the formation of propionic acid by strain 699 is not known. Enough strains of \textit{Actinomyces} have not been described to determine if the formation of this acid warrants any special taxonomic consideration.

It is commonly recognized that typical actinomycosis with formation of clubbed sulfur granules seldom occurs as a result of animal inoculations with known strains of \textit{A. israelii} (Rosebury, 1944). However, Hazen and Little (1958) have found that the young male golden hamster is suitable for testing the pathogenicity of \textit{Actinomyces} since infection occurs in a high per cent of these animals. Although strains 699 and OS 131 did not cause progressive disease in the hamster, Awerbach (1903) isolated a strain of \textit{Actinomyces} from a lacrimal infection which was pathogenic for a guinea pig. The guinea pig, which died 2½ months after intraperitoneal inoculation, had an encapsulated abscess with typically clubbed actinomycotic granules. Grüter (1933) inoculated dogs and guinea pigs with a strain which he named \textit{Actinomyces discofiliatus}. This strain, isolated from concretions in the lacrimal canal, caused abscess formation which healed after 12 to 18 days. Negroni (1938) considered \textit{A. discofiliatus} as the causal agent of a case of osteomyelitis of the mandible following a molar tooth extraction.

Comparison of strains 699 and OS 131 with several strains of \textit{A. bovis} isolated from “lumpy jaw” of cows showed strains 699 and OS 131 are distinct from \textit{A. bovis} in certain aspects of colony morphology and growth characteristics (Pine, unpublished data). Although differing from one another in several respects, strains 699 and OS 131 fall within the limits of characteristics described for \textit{A. israelii}. Smith (1953) also identified the strains isolated by him as \textit{A. israelii}. In his report, two of the strains isolated from the
larval canal which originally formed rough colonies formed smooth colonies after several transfers.

SUMMARY

Two strains of the genus Actinomyces have been isolated from cases of lacrimal canaliculitis and conjunctivitis of the eye. Although their colonies are smooth, these strains have cultural and morphological characteristics which would characterize them as Actinomyces israelii. However, one strain ferments glucose forming large amounts of propionic acid. Propionic acid was not formed by several other strains of Actinomyces studied previously.

REFERENCES