ORAL STRAINS OF ACTINOMYCES

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Bergey (1907) is generally credited with the first isolation of Actinomyces from the normal human mouth. Since then there have been a few reports of the recovery from the normal oral cavity of organisms which appear to have been identical, or nearly so, with strains isolated from clinical actinomycosis (Emmons, 1935, 1936, 1938; Ennever et al., 1951; Miller and Drake, 1951; Morris, 1954; Rosebury et al., 1944; Slack, 1942; Sullivan and Goldsworthy, 1940). In addition there have been numerous reports of the isolation from the mouth of one or more strains of organisms designated as Actinomyces. However, the data given in many of these reports were inadequate for the determination of the identity of the organisms described (Bartels, 1952; Bibby and Knighton, 1941; Bjerrum and Hansen, 1932; Citron, 1945; Crowley, 1941; Ennever and Warner, 1952; Garrod, 1952a, b; Geister and Meyer, 1951; Grythe, 1938; Harrison, 1948; Hemmens et al., 1941; Hemmens et al., 1946; Kay, 1947; Lord, 1933; Lord and Trevett, 1936; Meyer and Verges, 1950; Naeslund, 1925, 1926). More recently, Thompson and Lovestedt (1951) isolated organisms from the normal human mouth which differed significantly from those isolated from lesions of actinomycosis and suggested that these strains be designated as *Actinomyces naeslundii*. Garrod (1952b) has confirmed these findings. However, *A. naeslundii* has not yet been generally accepted as a valid species (Conant et al., 1954; Breed et al., 1957).

In order to characterize further oral strains of Actinomyces and to compare them with available strains isolated from lesions, approximately 200 of the former, and 11 of the latter have been studied critically, especially with respect to their morphological and cultural characteristics.

MATERIALS AND METHODS

All oral strains of Actinomyces were isolated from beef-extract-blood agar plates containing 0.1 per cent soluble starch and 1:1,000,000 basic fuchsin. Colonies were transferred to tubes of fluid thioglycolate medium (BBL) or thioglycolate containing 20 per cent horse meat infusion which were incubated at 37 C for 2 to 7 days, or rarely, for 10 days. After noting the type of growth obtained, all cultures were stained by Gram's method. Growth was then streaked on brain heart infusion agar (Difco) plates which were incubated at 37 C in an anaerobic jar in an atmosphere of 5 per cent CO_2 and 95 per cent N_2, as previously described (Howell and Pine, 1956). These plates were examined daily for 1 to 3 days at 100-fold magnification. If growth was evident, a sterile cover glass was placed over the microcolonies and the type of growth determined under a magnification of $\times$ 980 as described by Emmons (1935). The plates were returned to the anaerobic jar and incubation at 37 C continued for a total of 7 days, at which time colony characteristics were ascertained under 30-fold magnification, and the catalase reaction determined as previously described (Pine and Howell, 1956).

Inoculum for all further tests was grown in the liquid casitone starch medium containing 0.5 per cent glucose, adjusted to pH 6.5, under anaerobic seal described by Howell and Pine (1956). In order to obtain a uniform suspension, growth was homogenized, when necessary, by the use of a tissue grinder (A. H. Thomas no. 4288-B). Cells were washed three times in a 0.05 per cent aqueous solution of L-cysteine hydrochloride adjusted to pH 7.2 before sterilization at 121 C for 20 min. The washed cell suspension was then adjusted to an optical density of 0.500 $\pm$ 0.025 in a Beckman model B spectrophotometer in the same diluent (Howell and Pine, 1956). One drop of this suspension from a 1-ml pipette was used as the inoculum for fermentation tests; 0.1 ml per tube as inoculum for litmus milk or media containing bile, nitrate, or high concentrations of salt. In fermentation tests, 5 ml medium were used per tube, with all tests run in duplicate.

Carbohydrates were used in a final concen-

tration of 0.5 per cent, incorporated in the liquid casitone-starch medium adjusted to pH 6.5. These cultures were incubated without shaking for 7 days at 37 C under Na_2CO_3 + pyrogallol seals. The following carbohydrates were tested: glucose, maltose, lactose, sucrose, mannitol, salicin, raffinose, xylose, ribose, inositol, inulin, arabinose, melezitose, α-methyl-D-glucoside, α-methyl-D-mannoside, and glycerol. In the earlier experiments optical density (OD) was used as a measure of total growth (Beckman model B spectrophotometer at 660 mμ) and final pH determined with a Beckman model G pH meter. However, as a high degree of correlation was found to exist between amount of growth and final pH, in later experiments, final pH alone was used as an indication of the ability of a strain to utilize a given carbohydrate as well as a measure of acid production. A drop in pH of less than 0.49 was considered to be a negative reaction; a drop of 0.50 to 0.99 was considered to be a plus-minus reaction; and a drop of one pH unit or more was considered positive. As previously noted (Pine and Howell, 1956), no growth of these organisms occurs in this medium in the absence of a utilizable carbohydrate. Tolerance to salt and bile was determined by incorporating a total of 4 or 6.5 per cent sodium chloride or 10, 20, 30, or 40 per cent bile in fluid thioglycolate medium containing 20 per cent horse meat infusion, and incubating for 7 days at 37 C. Gelatin liquefication, nitrate reduction, and action on litmus milk were determined in the earlier experiments by methods previously reported (Pine and Howell, 1956). In later experiments, 0.1 per cent KNO_3 or 10 per cent gelatin were incorporated into fluid thioglycolate. Gelatin in horse meat infusion broth, and litmus milk cultures were incubated for at least 10 days either in anaerobic jars with 5 per cent CO_2 + 95 per cent N_2 or by sealing the tubes with Na_2CO_3 + pyrogallol. Voges-Proskauer and indole tests were performed on cultures which had been grown for 7 days in horse meat infusion broth or fluid thioglycolate.

For studies of oxygen tolerance or effects of increased carbon dioxide tension the liquid casitone-starch medium containing 0.5 per cent glucose was used. Five ml of medium were inoculated with 0.05 ml of the OD 0.500 suspension or with 0.05 ml of a 1:10,000 dilution of this suspension in 0.05 per cent aqueous cysteine hydrochloride. For incubation in air, the cotton plug was replaced with a metal cap; for incuba-

tion in air with increased carbon dioxide tension a Na_2CO_3 + KH_2PO_4 seal was used (Pine and Howell, 1956); and for anaerobic incubation the Na_2CO_3 + pyrogallol seal was used. All tests were run in duplicate, and all tubes were incubated on a rotary shaker at 37 C (Howell and Pine, 1956) for 7 days. All cultures were homogenized with a 5 ml pipette (Howell and Pine, 1956) prior to reading in the Beckman spectrophotometer or, if impossible to homogenize, growth was recorded as plus-minus, one, two, or three plus. In a few experiments, vitamin-free casein hydrolyzate (Nutritional Biochemicals Corporation) was used in place of casitone.

In addition to the strains isolated from the oral cavity in the absence of actinomycosis, 11 strains isolated from clinical actinomycosis and 4 additional strains used in previous studies (Howell and Fitzgerald, 1953; Howell and Pine, 1956; Pine and Howell, 1956) were included for comparative purposes. The origin and source of the latter 2 groups are shown in table 1.

Eleven oral strains and two from lesions (strains 277 and 287) were tested for pathogenicity in 21 to 25-day-old male golden hamsters by intraperitoneal inoculation. Growth suspensions were prepared in physiological saline, 0.05 per cent aqueous L-cysteine hydrochloride, or liquid casein hydrolyzate-starch medium containing 0.5 per cent glucose (Howell and Pine, 1956). All suspensions were standardized to an OD of 1.0 and 0.5 ml was injected per animal intraperitoneally. In one experiment the inoculum, suspended in cysteine hydrochloride solution, was mixed with an equal volume of 5 per cent hog gastric mucin suspension (Wilson type 1701W); in another, 0.5 ml of mucin suspension per animal was injected intraperitoneally approximately 4 hr prior to injection of the organisms; and in a third experiment the organisms suspended in saline were injected alone. Animals were sacrificed at intervals of 7 to 42 days.

RESULTS

On the basis of type of growth of initial cultures in fluid thioglycolate medium and morphology of micro- and mature colonies on brain heart infusion agar, two major types of oral Actinomyces were isolated. For convenience, these will be described as types 1 and 2.

A total of 42 strains of type 1 are included in this study. Of this number, 31 were isolated from blood agar plates which had been incubated

TABLE 1
Origin and source of stock strains of Actinomyces

| Strain No.	Source	Donor
260	Gingival scrapings	Dr. Ada Clark and Dr. Arthur Ellison
261	Gingival scrapings (?)	Dr. Ada Clark and Dr. Arthur Ellison
262	Cervico-facial actinomycosis	Dr. Ada Clark and Dr. Arthur Ellison
263	Cervico-facial actinomycosis	Dr. Ada Clark and Dr. Arthur Ellison
277	Brain abscess	Dr. Luther Thompson
279	Sinus following tooth extraction	Dr. Luther Thompson
281	Abscess of cheek	Dr. Per Holm
286	Gingival scrapings	Dr. R. J. Fitzgerald
287	Cervico-facial actinomycosis	Dr. Arthur Ellison
295	Human pleural fluid	ATCC no. 10048
296	Atypical actinomycosis	ATCC no. 10049
348	Cervico-facial actinomycosis	Dr. Esther Meyer
350	Cervico-facial actinomycosis	Dr. Philip O. Nice
353	Blood stream following tooth extraction	Miss Sylvia King
356	Generalized actinomycosis	Dr. N. F. Conant and Dr. Leo Pine
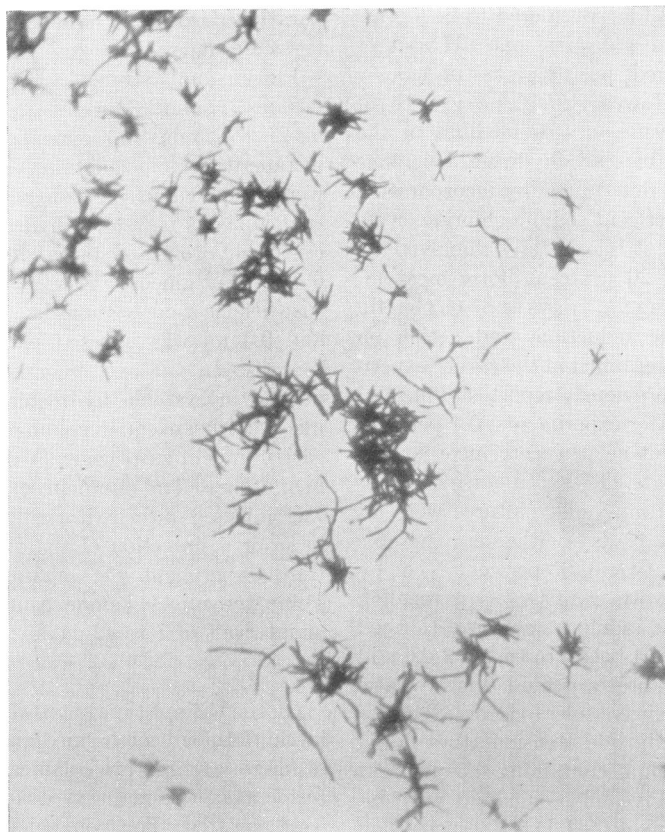
Figure 1. Smear of type 1 strain (*Actinomyces naeslundii*) from thioglycolate medium. Gram stain. ×650.
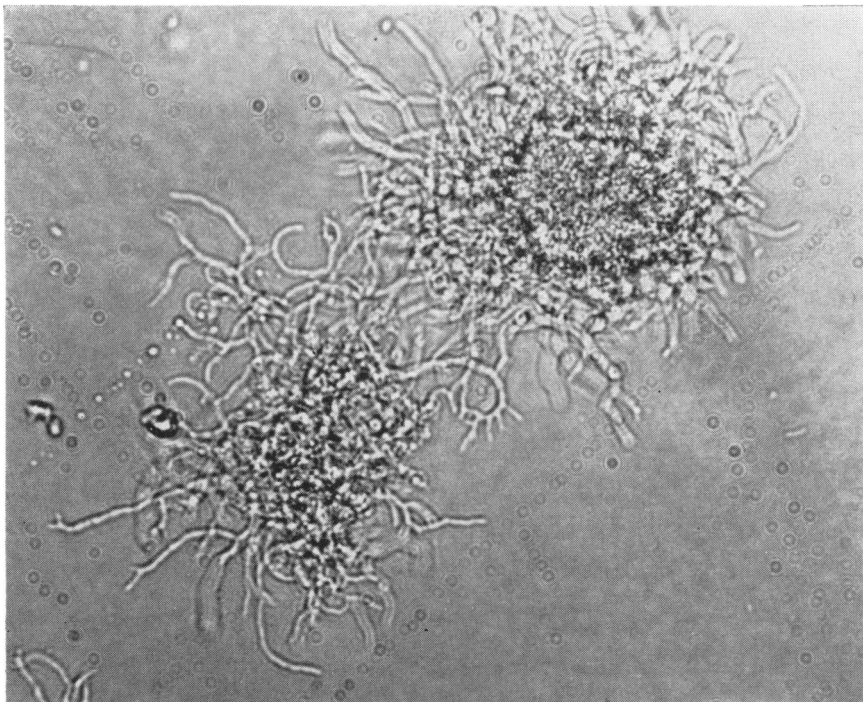
Figure 2. Microcolony of type 1 strain (*Actinomyces naeslundii*). Twenty-four hr on brain heart infusion agar incubated anaerobically with 5 per cent CO_2 + 95 per cent N_2. ×750.
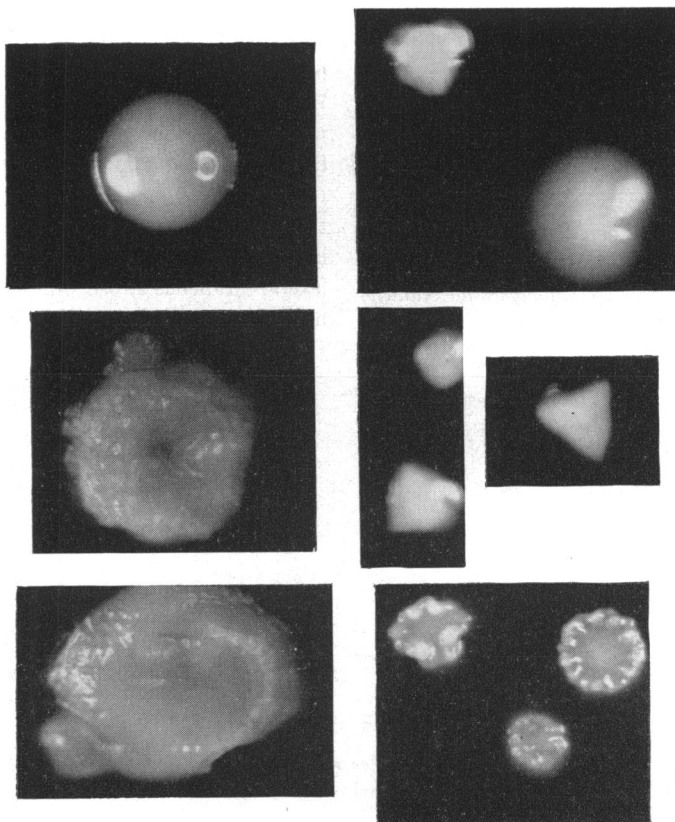

Figure 3. Mature colonies of several strains of type 1 (*Actinomyces naeslundii*). Seven days on brain heart infusion agar incubated anaerobically with 5 per cent CO_2 + 95 per cent N_2 at 37 C. ×23.

aerobically. Colonies on the isolation plates were quite variable. The majority were white, < 0.5 mm, raised to hemispherical with a ground-glass to finely lobate surface, and a flat, filamentous edge. Some were hemispherical and smooth with a clear, flat, narrow periphery resembling a derby hat. A few were tenacious, but most were not. By

Figure 4. Growth and concomitant acid production of type 1 strains (Actinomyces naeslundii) on specified carbohydrates.

transmitted light they were translucent or opaque. Others were 1 mm or greater, creamy white, and either convex or heaped. The latter were usually lobate or spiked, and occasionally showed a central crater. The convex colonies were either smooth or granular and pitted. Both of these types sometimes had a flat undulate or irregular periphery; both were creamy and soft, and either translucent or opaque.

When first isolated, growth in fluid thioglycolate medium developed rapidly, within 24 to 48 hr; it usually consisted of a heavy flocculent mass filling the upper portion of the medium; in a few strains growth was flocculent and granular or granular alone.

Gram-stained smears of thioglycolate cultures usually showed filamentous gram-positive organisms and, occasionally, rod forms (figure 1). The filaments were slender and of variable thickness with either straight, clavate, or tapered ends. They were solidly stained, segmented, beaded, or stippled. In a few cultures, only gram-positive rods were found. These were slender, long or short, curved, straight, or bent. Occasionally, clubbed forms occurred.

Microcolonies, after 18 to 24 hr incubation anaerobically on brain heart infusion agar, sometimes showed moderately long, slender, branching filaments similar to those seen in type 2 (figure 7), but usually showed a dense mass of diphtheroid cells or a dense clump of filaments surrounded by a somewhat rudimentary type of mycelium (figure 2). Frequently the hyphae were curved; the branches were uneven in diameter and arose in a briar-like manner. Often 2 or 3 short, stubby, lateral branches arose on one side of a single main filament. After 5 to 7 days incubation ma-
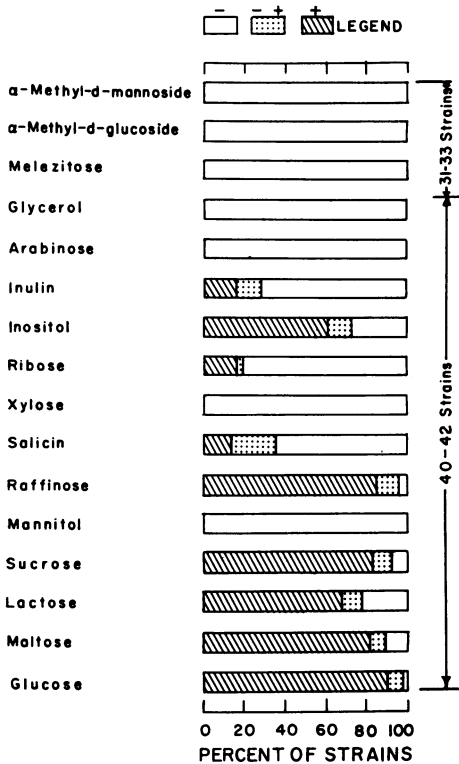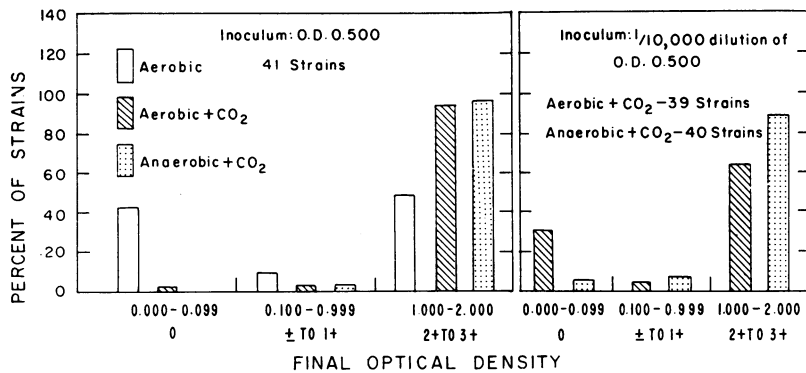

Figure 5. Growth of type 1 strains (Actinomyces naeslundii) aerobically, aerobically with increased carbon dioxide tension, and anaerobically with carbon dioxide.
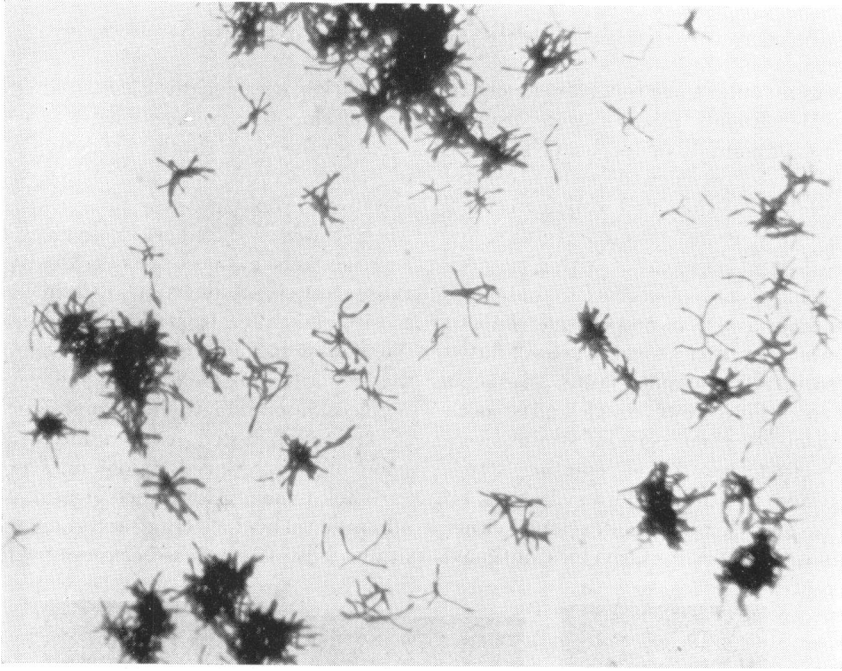
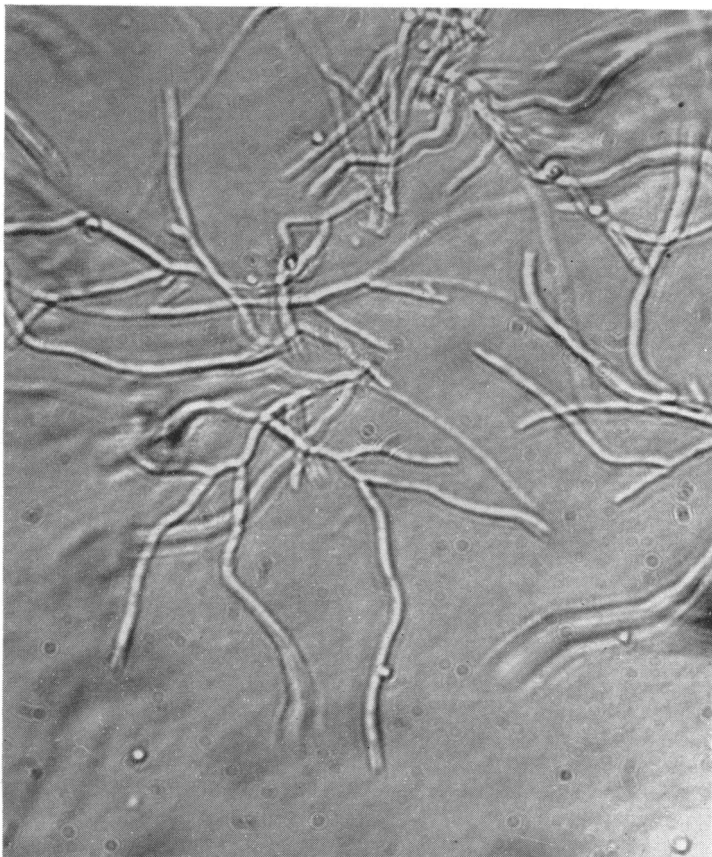*Figure 6.* Smear of type 2 strain (*Actinomyces israelii*) from thioglycolate medium. Gram stain. ×650

Figure 7. Microcolony of type 2 strain (*Actinomyces israelii*). Forty-eight hr on brain heart infusion agar incubated anaerobically with 5 per cent CO_2 + 95 per cent N_2 at 37 C. ×750.

ture colonies were found to be rough or smooth or both (figure 3). Rough colonies were usually small, less than 1 mm in diameter, heaped, rugose or irregularly lobate, occasionally with a central crater. Smooth colonies reached a diameter of 2 to 3 mm; they were usually round and entire, low convex, smooth, and transparent with a tiny optically dark central core, or convex to umbonate or pulvinate, finely granular, and opaque. Smooth convex colonies frequently showed a raised, fluted margin. Occasionally, colonies were triangular or irregular in outline. Both rough and smooth colonies developed when certain strains were plated for the first time. Upon replating, either colonial type gave rise to both forms observed on the original plate. Other strains developed either rough or smooth colonies exclusively. Continued cultivation occasionally resulted in the production of smooth colonies alone.

All strains were catalase negative. The ability of type 1 strains to grow and produce acid on specified carbohydrates is presented in figure 4. Most strains produced acid from glucose, maltose, sucrose, and raffinose; about three fourths of those tested produced acid from lactose and inositol; and a few from salicin, ribose, and inulin. None grew in, or produced acid from, mannitol, xylose, arabinose, melezitose, glycerol, α-methyl-D-glucoside or α-methyl-D-mannoside. Thirty-six of 41 strains tested reduced nitrate to nitrite; none of 37 produced indole or acetyl-methylcarbinol; none of 38 liquefied gelatin. Nineteen of 40 produced acid, clotting, and some reduction of litmus milk; 16 produced acid only with some re-
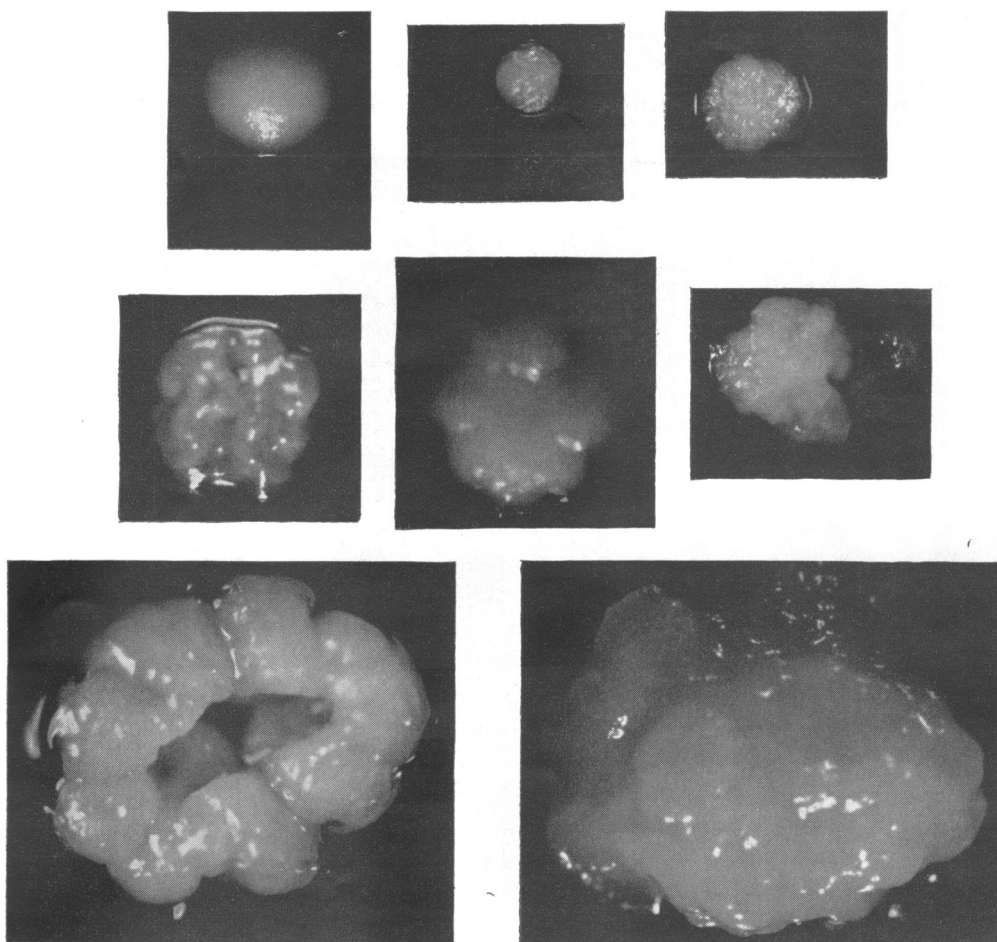

Figure 8. Mature colonies of several strains of type 2 (*Actinomyces israelii*). Six to 7 days on brain heart infusion agar incubated anaerobically with 5 per cent CO_2 + 95 per cent N_2 at 37 C. ×23.

duction; 5 gave no reaction. Eight of 28 gave some growth in fluid thioglycolate medium containing 4 per cent sodium chloride, but none grew in 6.5 per cent salt. Seventeen of 28 grew in fluid thioglycolate medium containing 10 per cent bile, 15 in 20 per cent bile, 11 in 30

per cent bile, but only 3 of 27 grew in 40 per cent bile.[1]

Oxygen tolerance studies (figure 5) indicated that, when grown on the casitone-glucose starch medium, this group of organisms was facultative, but increased carbon dioxide tension was required for good growth in air. With a very small inoculum, more strains grew better when grown anaerobically with carbon dioxide than aerobically, but with 64 per cent an optical density of 1.00 or greater was obtained when grown in air with increased carbon dioxide tension.

Suspensions of 3 strains were used to inject a total of 28 animals by various techniques. In 2 animals, 1 sacrificed after 8 days, the other after 14 days, a solitary nodule approximately 1 mm in diameter was found on the peritoneal lining of the body wall. Actinomycetes were demonstrated in smears from each lesion.

On the basis of the type of growth in thioglycolate and colonial morphology, strains 260, 263, 279, and 286, used in previous studies, were similar to type 1 organisms. In addition, they fermented only glucose, maltose, lactose (except for strains 279 and 286), and raffinose, and in the case of strains 279 and 286, inositol. Salicin was also fermented by strains 279 and 286. All 4 strains reduced nitrate to nitrite. Strains 260 and 279 were relatively oxygen tolerant, with good growth in air with CO_2 resulting from an inoculum of the 1:10,000 dilution of the OD 0.500 suspension; strain 263 was less oxygen tolerant, growing in air with CO_2 only with the original OD 0.500

[1] Bile and salt media were used at the suggestion of Morrison Rogosa in the expectation that these would aid in the differentiation of strains of Actinomyces from "anaerobic diphtheroids."

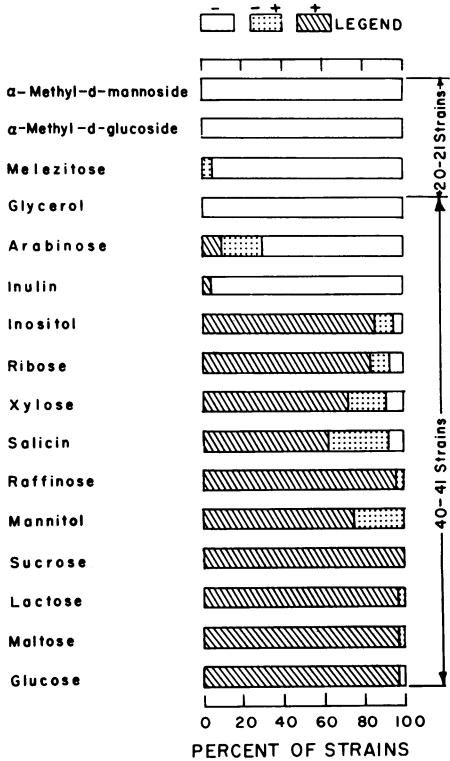Figure 9. Growth and concomitant acid production of type 2 group 1 strains (Actinomyces israelii) on specified carbohydrates.
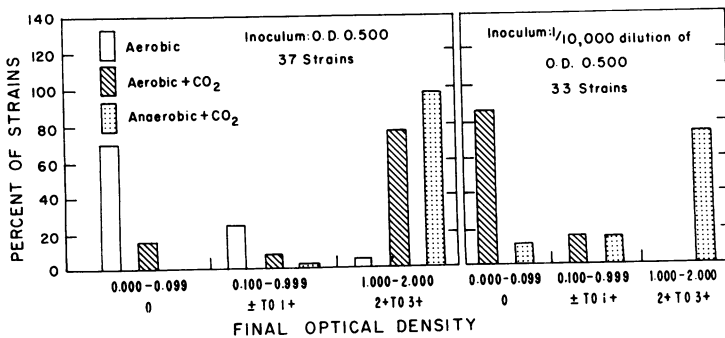

Figure 10. Growth of type 2 group 1 strains (Actinomyces israelii) aerobically, aerobically with increased carbon dioxide tension, and anaerobically with carbon dioxide.

suspension. However, when strain 279 was grown in the same medium prepared with casein hydrolyzate in place of casitone, it failed to grow in air with CO_2 at a 1:1000 dilution of the OD 0.500 suspension, whereas good growth was obtained in the casitone medium with this inoculum.

Figure 11. Growth and concomitant acid production of type 2 group 2 strains (*Actinomyces israelii*) on specified carbohydrates.

All organisms of type 2 were isolated from blood agar plates which had been incubated anaerobically with 5 per cent CO_2 + 95 per cent N_2.

The majority of the colonies of this type were immature on the isolation plates. In general, they were <0.5 mm in diameter, flat to low convex, round or oval, gray, with a granular surface and filamentous edge. Occasionally they were heaped in the center. Others ranged from low convex to heaped and were finely lobate, sometimes surrounded by a clear, flat peripheral zone, or had irregular processes extending from the center of the colony. In a few instances the colonies were well isolated and mature. Such colonies were 0.5 mm or greater in diameter, heaped, glistening, white, and coarsely lobate or raspberry-like, with a somewhat irregular edge. All colonies were opaque by transmitted light and often made depressions in the agar.

Upon transfer from the isolation plate to fluid thioglycolate medium, the majority of strains grew slowly as discrete, variably sized, lobulated granules, with about one third growing as tiny discrete granules. Gram-stained smears of such cultures usually showed slender, gram-positive rods which varied considerably in length (figure 6). These were straight, curved, or bent at an angle, quite often appearing in Y, V, or T formation. Usually a few clubbed or clavate forms were present. Filaments were occasionally seen; when present, these were straight or sinuous, with straight or clavate ends, solidly stained or beaded.

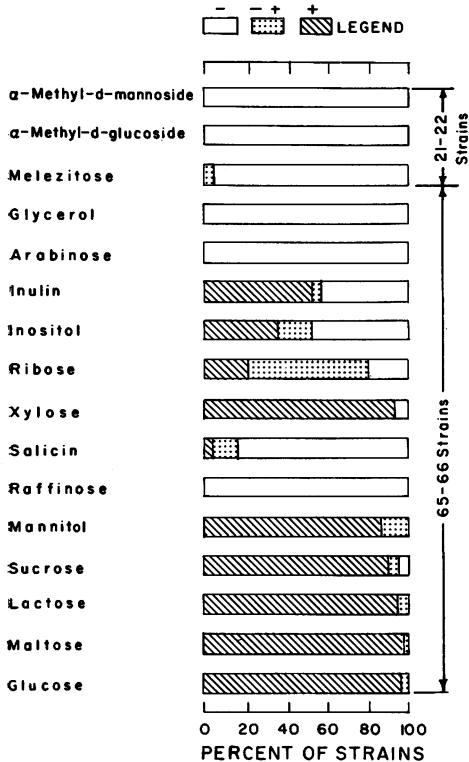All strains, in microculture on brain heart infusion agar, produced a well developed
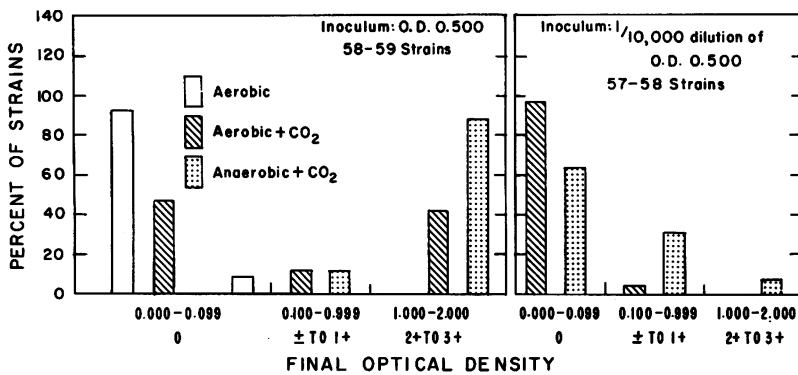

Figure 12. Growth of type 2 group 2 strains (*Actinomyces israelii*) aerobically, aerobically with increased carbon dioxide tension, and anaerobically with carbon dioxide.

branching mycelium (figure 7), usually within 48 hr. The filaments were slender, of variable length, straight or slightly tortuous, nonseptate, and freely branched, with the branches arising singly and at an acute angle.

Mature colonies were quite variable (figure 8). They were usually 0.5 mm or less in diameter, but well isolated colonies sometimes reached 1 mm or more. They were round, entire, strongly convex to pulvinate with a finely granular or pitted surface; heaped, rounded and finely lobate; heaped, cerebriform to coarsely lobate with an irregular, lobate edge; very heaped, bread-crumb or cauliflower-like; or irregular, very heaped, raspberry-like with or without a central crater. They were usually creamy white in color, though occasionally grayish-white colonies developed.

All were catalase negative. None of 122 strains tested liquefied gelatin, although growth in this medium was often poor; 4 of 100 grew in medium containing 4 per cent sodium chloride, but none in 6.5 per cent; none of 95 grew in medium con-
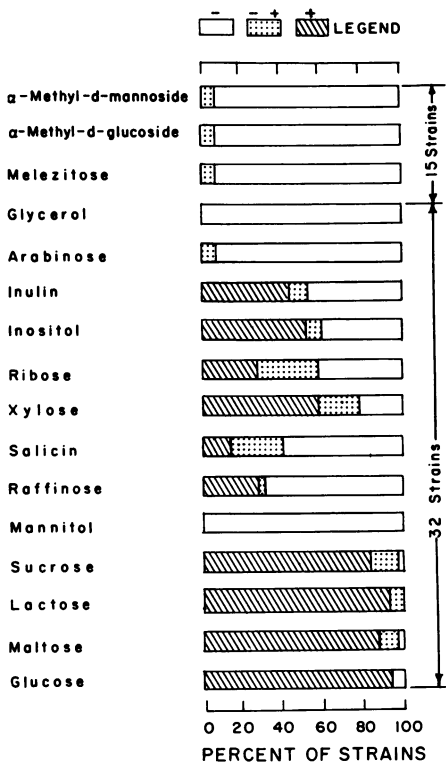

Figure 13. Growth and concomitant acid production of type 2 group 3 strains (*Actinomyces israelii*) on specified carbohydrates.
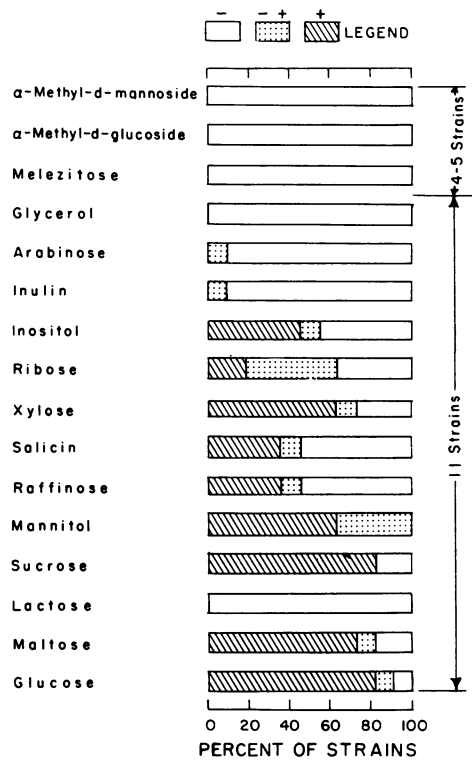

Figure 14. Growth and concomitant acid production of type 2 group 4 strains (*Actinomyces israelii*) on specified carbohydrates.

taining 10 per cent bile; none of 143 produced indole; and only 6 of 133 produced acetyl-methylcarbinol. Almost all strains grew in, and produced acid from, glucose, maltose, and sucrose. On the basis of growth in, and fermentation of, other carbohydrates it was found that this group of 158 strains could be subdivided into 5 subgroups (figures 9, 11, 13 to 15). Subgroup 1, composed of 41 strains (figure 9), was very active, producing acid from lactose, mannitol, raffinose, salicin, xylose, ribose, and inositol; occasional strains fermented arabinose, one inulin, and none the other carbohydrates tested. Half of the strains reduced nitrate to nitrite; half gave no reaction in litmus milk; 4 produced slight acid, and the remainder slight reduction only.

Organisms of this type were considerably less oxygen tolerant than type 1 strains (figure 10), although with a large inoculum (OD 0.500) 75 per cent of the strains produced an OD of 1.00 or greater when grown in air with increased carbon dioxide tension. With a small inoculum, none
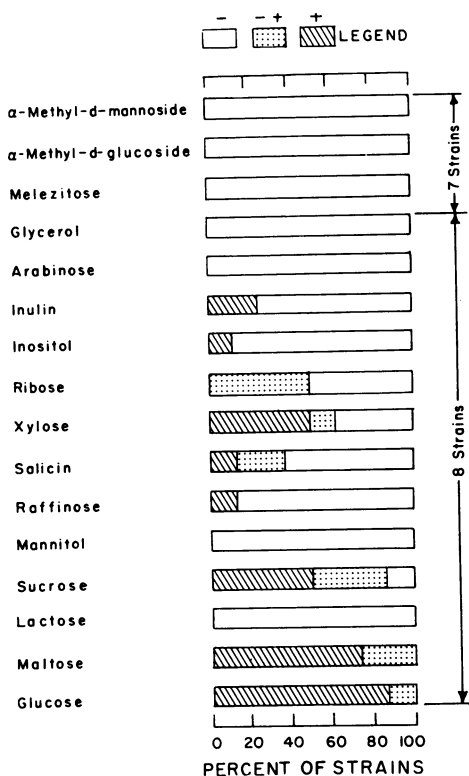
Figure 15. Growth and concomitant acid production of type 2 group 5 strains (*Actinomyces israelii*) on specified carbohydrates.

grew well except when cultivated anaerobically with carbon dioxide.

Stock strains 261, 262, 277, 281, 287, 295, 296, 353, and 356 were similar, on the basis of fermentations, to organisms of this subgroup. Five of the stock strains reduced nitrate to nitrite, 4 produced acetyl-methyl-carbinol, none produced indole, and 1 produced an acid clot in litmus milk, the others producing no reaction on this medium. All except strain 353 grew well in air with increased carbon dioxide tension when a large inoculum was used (OD 0.500), but all grew only anaerobically with carbon dioxide when a small inoculum was employed (1:10,000 dilution of OD 0.500).

A second subgroup, composed of 66 strains, grew on and fermented lactose, mannitol, and xylose, but not raffinose. Some fermented ribose, inositol and inulin, with relatively poor, moderate, and good growth, respectively. Practically none utilized salicin or the other sugars tested (figure 11). Thirty-four of the 66 reduced nitrate;

23 produced acid with some reduction in litmus milk, 39 an acid clot. One stock strain, 350, was similar to organisms of this subgroup.

Strains of subgroup 2 were much less tolerant of oxygen than those of subgroup 1. Only about 40 per cent produced good growth in air with increased carbon dioxide tension from a large inoculum; growth was poor with a small inoculum, even under anaerobic conditions (figure 12). No growth of the stock strain occurred, even with large inoculum, in air with increased carbon dioxide tension.

A third subgroup, consisting of 32 strains, failed to grow with mannitol as a substrate (figure 13). These strains grew in and produced acid from lactose; most utilized xylose; about half, ribose, inositol, and inulin; approximately one-third produced acid from raffinose and salicin. Other carbohydrates tested were not utilized. Thirteen of 31 strains reduced nitrate; 10 of 29 produced acid only in litmus milk, 4 acid with some reduction, and 12 an acid clot with reduction.

Stock strain 348 behaved similarly to this group. No growth of this strain occurred in air with increased carbon dioxide tension.

Eleven strains failed to grow in lactose (figure 14). Most of these produced acid from mannitol; many from xylose, ribose, and inositol; about half from raffinose and salicin, with the other substrates not being utilized. Four strains reduced nitrate; 4 produced acid or acid with some reduction in litmus milk, 5 were negative, and 1 gave an acid clot with reduction.

A small group of 8 strains failed to utilize both lactose and mannitol (figure 15). Five of them produced acid from xylose, 4 from ribose, 3 from salicin, 2 from inulin, and 1 from raffinose and inositol. Three of 7 strains reduced nitrate; 1 produced acid from litmus milk, 2 an acid clot with reduction, and 4 were negative.

Oxygen tolerance studies of subgroups 3 to 5 gave results similar to those obtained in subgroup 2.

A total of 86 hamsters were injected in various ways with 7 strains of organisms of subgroup 2; 5 with stock strain 277 and 5 with strain 287. No disseminated or progressive disease was found in any animal. Nine, sacrificed after 8 to 42 days, showed solitary nodules 1 to 2 mm in diameter within the peritoneal cavity from which the organisms were recovered on smear or culture.

DISCUSSION

Breed et al. (1957) define the genus *Actinomyces* as follows: True mycelium produced. The vegetative mycelium fragments into elements of irregular sizes and may exhibit angular branching. No conidia produced. Not acid-fast. Anaerobic to microaerophilic. Pathogenic for man and other animals.

On the basis of microscopic morphology of young colonies, with subsequent breaking up of the filaments into irregular bacillary or diphtheroid cells, the absence of conidia, and the lack of acid-fastness, it would seem justifiable to include both type 1 and 2 organisms described above in the genus *Actinomyces*. Although the mycelium produced by type 1 organisms is generally quite limited in extent (figure 2), it must be regarded as a true mycelium. All were gram-positive and catalase negative.

Many of the oral strains of type 1 grew either aerobically or aerobically with increased carbon dioxide tension. However, it was shown that aerobic growth is dependent upon both the size of the inoculum and the medium employed. Preliminary studies with similar strains indicated that, had a medium employing vitamin-free casein hydrolyzate been used in place of casitone, the percentage of strains growing aerobically would have been considerably reduced (Howell, *unpublished data*). Therefore, whether or not an organism is considered to be an aerobe or a microaerophile depends upon the conditions employed for the test. None of the organisms studied here could be considered to be aerobes, even though some appeared to be facultative under certain of the conditions used.

Type 1 organisms appear to be identical with those described by Thompson and Lovestedt (1951) for which the name *Actinomyces naeslundii* was proposed to distinguish them from the pathogenic species, *Actinomyces israelii*, and *Actinomyces bovis*. Strains of *A. naeslundii* differ from *A. israelii* in that they may be isolated more frequently from blood plates incubated aerobically, rather than anaerobically; growth in thioglycolate is usually rapid and diffuse or flaky; the mycelium on brain heart infusion agar is primitive, reduced in amount, and usually of a briar type with uneven branching; and mature colonies within a given strain may be rough or smooth, or both. They do not ferment mannitol or xylose, but usually ferment raffinose; nearly all strains reduce nitrate to nitrite; many grow in media containing 10 to 20 per cent bile; most strains are relatively oxygen tolerant.

Strains of *A. bovis* Harz were not included in the present study as no organisms of this type were isolated from the oral samples. However, from Thompson's description of this organism (Thompson, 1950) and a preliminary study of one of his strains, it differs from type 1 strains in that all strains of *A. bovis* are strict anaerobes which produce only smooth colonies, differing from most of the smooth colonies produced by *A. naeslundii*, and in that most were isolated from bovine sources.

Type 1 organisms differ from species of the genus *Nocardia* in that they are catalase negative and facultative or microaerophilic. The mycelium, as well as colony formation, differs from that of typical strains of Nocardia.

It is recommended, therefore, that the name *Actinomyces naeslundii* (*A. naeslundi*, Thompson and Lovestedt, 1951), be adopted for organisms similar to those described herein as type 1 *Actinomyces*.

Type 2 organisms were identical in nearly all major respects with 10 strains isolated from human lesions. On the basis of growth in thioglycolate, the well developed branching mycelium produced in young cultures on brain heart infusion agar incubated anaerobically, and the morphology of mature colonies, these strains appear to be typical of the organisms described as *Actinomyces israelii* (Kruse) Lachner-Sandoval (Breed et al., 1957). This organism has also been described by various investigators as *Actinomyces bovis* Harz, but in view of the works of Erickson (1940), and particularly of Thompson (1950), it seems fairly certain that the name *A. bovis* should be reserved for the bovine type of organism, and *A. israelii* for the type usually isolated from human lesions.

Many of the type 2 oral strains and some of those isolated from lesions when grown on the casitone medium with a large inoculum, gave good growth when incubated aerobically with increased carbon dioxide tension. However, when the inoculum was decreased, or in the case of strains 281, 295, and 296, when casein hydrolyzate was substituted for casitone, growth took place only when grown anaerobically with carbon dioxide. Thus, although these strains were much less oxygen tolerant than those of type 1, aerobic

growth of type 2 strains was similarly dependent upon the size of the inoculum and the composition of the medium. A single strain isolated from a lesion, tested for oxygen tolerance within a very short time after its isolation, grew aerobically on the casitone medium with increased carbon dioxide tension when a large inoculum was used. From the results obtained, all strains of type 2 were regarded as being essentially microaerophilic rather than facultative organisms.

Previous investigators (Holm, 1930; Negroni and Bonfiglioli, 1937; Erickson, 1940; Sullivan and Goldsworthy, 1940; Slack, 1942; Ennever et al., 1951; Miller and Drake, 1951) have reported that A. israelii, or similar strains, ferment glucose, maltose, sucrose, and lactose with the production of acid but no gas; most have reported acid production from mannitol and some from raffinose and xylose. In the studies reported here it was found that this type of organism, on the basis of study of approximately 160 strains, could be divided into 3 well defined groups, with a few additional strains falling into 2 other groups. Nearly all fermented glucose, maltose, lactose, and sucrose. Two of the 3 major groups produced acid from mannitol, and 1 of the 2 produced acid from raffinose. However, as these groups were essentially alike in other major characteristics, separation on the basis of fermentation alone would seem to be unwarranted.

The findings of Hazen et al. (1952) and Hazen and Little (1958), that young male golden hamsters are suitable animals for testing pathogenicity of Actinomyces bovis (A. israelii), was not confirmed in this study. However, it seems probable that a much smaller dose for inoculation was used in these experiments than in those reported by Hazen. This may account for the variable results obtained.

Attempts to secure strains of Actinomyces odontolyticus described by Batty (1958) for comparison with the oral strains were unsuccessful.

SUMMARY

A comparison has been made of 200 strains of Actinomyces isolated from the oral cavity in the absence of actinomycosis, and 11 isolated from actinomycotic lesions. It was found that the oral strains were of two main types, one corresponding to the organisms described previously for which the name Actinomyces naeslundii was proposed, and the other essentially identical to those isolated from lesions, which should be designated as Actinomyces israelii. It has been recommended that A. naeslundii Thompson and Lovestedt be accepted as the proper specific name for the fast growing facultative type of Actinomyces.

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