SPIROCHAETAL AND MYCOLOGIC ASPECTS OF THE ORAL FLORA WITH SPECIAL REFERENCE TO VINCENT'S INFECTIONS

by

JAMES WILLIAM BROOKE

A Thesis

Presented to the Department of Bacteriology
and the Graduate Faculty of the University of Oregon
in partial fulfillment of the requirements for the degree of
Master of Arts

June 1938

APPROVED:

[redacted]

Major Adviser

[redacted]

For the Graduate Committee

of the University of Oregon

Medical School

TABLE OF CONTENTS

PART I Review of literature since 1952

Introduction 1

Organisms isolated from normal mouth 2

Bacterial picture in disease 3

Mycologic aspects 13

Summary 19

Tables and charts 20

References 24

PART II Regional Study of bacteria in clinically normal mouths 29

Methods 34

Results 35

Summary 39

References 40

Tables 42

PART III Mycotic gingivitis--a case report 45

Laboratory study of organism 47

Tables 50

I

REVIEW OF LITERATURE SINCE 1935

Introduction

In the preparation of a previous paper (1) we were impressed both by the amount of work which had been done on the oral flora and by the apparent lack of any attempt to assemble and correlate the published observations. Recent technical advances, the development of routines for antigenic analysis, studies on variants, the concepts of latent virulence, and the discovery of organisms having long latent incubational periods have all indicated that a re-evaluation of the flora of the mouth is desirable. In this communication we shall discuss the pathogens and non-pathogens which occur in a relatively high incidence in the clinically normal mouth as recorded in the literature of the last six years. Particular attention is devoted to the mycological aspects of the flora. It is our contention that fungous diseases, systemic as well as local, will achieve a much greater significance as more knowledge of their characteristics is obtained. Pasteur's epoch-making discoveries distracted attention fromthis field and it has only been in the last few years that their role in the production of disease has become partially appreciated.

Organisms isolated from the normal mouth

Witkowski (2) in the mouths of fifty parturient women found Staphylococci present in 100 per cent and non-hemolytic streptococci present in 90 per cent. Table One shows the per cent incidence of the organisms found in the mouths of new born children and in the mouths of the mothers of the same children. These organisms were determined by cultural methods which employed primary 24-hour incubation in lactose broth tubes and subsequent subculturing. Other investigators who incubated their tubes only five or six hours obtained a higher per cent of sterile mouths among new-born children and Witkowski offers this difference in incubation time as an explanation.

Fleming and Maclean (3) investigated the incidence of the influenza bacillus in the mouths of normal people. They found that this gram negative, non-motile, pleomorphic bacillus grew profusely on boiled blood agar, formed but pin-point colonies on simple blood agar, and developed not at all on plain agar. Whereas the incidence of influenza bacilli had been reported variably by different investigators, these two workers were able to recover it from all thirty of the mouths they examined.

They found that Hemophilus influenzae would grow well on a blood agar plate if near a colony of staphylococci but not on a simple agar plate under the same conditions. H. parainfluenzae would grow in both places. These investigators concluded that for

the growth of H. influenzae two factors, a V and an X, were necessary. The X factor,, or hemtin, was present in autoclaved blood agar and the V factor, a thermolabile substance, was elaborated by many bacteria, particularly the staphylococci.

From fourteen mouths which were either clinically normal or the site of paradentosis, Hewitt (30) obtained forty-seven different strains of aciduric bacteria which were culturally and biochemically different. These large, gram-positive, non-motile bacilli did not maintain their serological specificity in the course of two years growth on artificial media and she suggests that one ought to consider the mouth as a normal harbor for a constantly present group of aciduric bacteria, the individual components of which are a matter of academic interest only.

The incidence of certain bacteria in normal and diseased mouths was investigated by Wohlfeil and Schulz (4). Table 2 summarizes their findings. These authors believed that the low pH associated with caries would explain the lowered incidence of hemolytic streptococci in mouths presenting carious teeth. It is known that acidity inhibits the growth of streptococci. These men further find and conclude that the lacto-bacillus is not found oftener in anginal cases than in healthy mouths. Gundel and Linden (5) found the gram-negative cocci, non-hemolytic streptococci, group IV pneumococci to be the predominant organisms in the mouths of 60 healthy school children. It is their belief that the school children constitute a reservoir for the agent of influenza.

Table 3, from Brooke, shows the organisms encountered in a purely morphologic study of the mouth. This illustrates the incidence of the various morphologic groups, from a consideration of which the possible individual species may be deduced.

Smith's monograph (6) on the oral spirochaetes sums up most of the literature pertaining to this filed prior to 1933. Only supplemental information is added here.

From these recent contributions, revising and extending the older concepts of the oral flora, one is brought to a new appreciation of the pathogenic potentialities of the oral bacterial inhabitants.

BACTERIAL PICTURE IN DISEASE

The bacterial picture under pathological conditions is extremely varied. One must consider not only the conversion of potential pathogens into active ones, but also the invasion of extrinsic organisms which may or may not have an etiological role in the production of the disease picture. The very multiplicity of the organisms present normally, it being apparent that numerous pathogens are included, makes difficult an exact evaluation of the part the organisms play. Several possibilities present themselves: (1) symbiotic pathogenesis with two or more organisms supplementing one another, (2) primary pathogenicity with one organism solely responsible, (3) saprophytic secondary invasion of a lesion produced

by traumatic, metabolic, or other causes, (4) abandonment of latent virulence of the secondary invaders as a direct result of improved growth conditions afforded by some primary disturbing cause and consequent domination of the bacterial picture. The skepticism which Arnold and Stuart (7) express about the value of a study of the oral flora may well be appreciated if these possibilities are borne in mind. Any lesion in the mouth may be, per se, regarded as secondarily infected and the determination of the primary etiological agent is difficult.

Owing to the present tendency to label as a Vincent's infection any oral lesion which is characterized by pain, bleeding, and the formation of a moderately crateriform ulcer and to the fact that such a diagnosis is commonly supported by the finding of fusiform bacilli and spirochaetes in slide preparations from such lesions, these same organisms have received much attention. Currently there seems to be a doubt as to the value of the slide diagnosis of Vincent's infections and recently other suggestions (8, 9, 10, 31) pertinent to the etiology of this disease have been suggested. Since Vincent's infections probably attract the greatest interest in a study of oral lesions---it is well to start with a consideration of the organisms supposedly causative of that disease. Smith's monograph is a comprehensive review of the literature on the subject and no attempt is here made to do more than supplement

it with reviews of papers since it's publication date.

Broughton-Alcock (11) reports the finding of spirochaetes in histological sections prepared from an asymptomatic nodule taken from the inner margin of the lower lip of an aged healthy adult. The tissue was fixed in formol-saline and stained with haematoxylin-eosin, Van Gieson's and the Gram stains. Spirochaetes were found in the Malpighian layer. The epithelium appeared intact. Morphologically according to the investigators, this organism resembled the Treponema macrodentium. It was gram positive, probably, he says, due to insufficient decolorization. In the corium about the organisms there was a polymorphonuclear infiltration. He concludes, after quoting other investigators who have reported instances of the invasion of the intact buccal membrane by spirochaetes:

"I suggest that some normally saprophytic spirochaetes of the buccal area and alimentary tract in their unceasing butting and boring on the epithelial lining may occasionally pass inward between the cells only to be destroyed in progress. In the specimen shown the spirochaete appears to have passed through and stimulated an inflammatory reaction in the body defence. When other damaging influences on the mucosa, such as toxins from other micro-organisms, commensalism, symbiosis, or trauma

act in association and lead to lowered tissue defence, some spirochaetes may resist this destruction and acquire relative or complete pathogenesis."

It has been suggested that Vincent's infection is merely a manifestation of an otherwise subclinical scurvy and that the fusiform bacilli and spirochaetes are secondary saprophytic invaders. This is readily intelligible on the basis of the theory that deficiency of the intercellular cement substance accounts for the hemorrhages characteristic of the scorbutic syndrome (12). A deficiency in this substance would allow the spirochaetes in their "unceasing butting and boring" as Broughton-Alcock suggests, ready passage through the buccal membrane. Pettit (8) asserts that the fusiform bacilli and spirochaetes are merely secondary invaders in a lesion primarily produced by a vitamin C or A deficiency.

Tunnicliff (13) found spiral forms in some of her cultures of the fusiform bacilli and advanced the hypothesis that these were merely variants of the fusiform bacilli. She found, moreover, that the addition of certain chemicals to the culture media would stimulate the formation of filamentous and spiral forms in these cultures. Two strains of the gram-negative non-motile fusiform bacilli over a culture period of three years failed to show any development of motile forms except when grown on malt extract sheeps blood agar. After studying the colony formation

she concluded that the smooth colonies represented the bacillary forms while the rough colonies were composed chiefly of motile variant forms. It is her contention that some of the oral spirochaetes heretofore described are merely dissociants of the fusiform bacilli.*

Woodburne has described some interesting lesions of the oral cavity which present a different bacterial picture. In streptococci hypertrophic gingivitis the clinical picture is somewhat similar to that of Vincent's infection. Clinically the gums are inflamed, swollen, sore and tender with extension of the tenderness to the mucosa of the cheeks and hard palate. Interproximally there is protrusion of the edematous gum tissue. The gums have a bright red, rolled, semi-translucent border but there is no ulceration, erosion, pustulation, or vesiculation. The temperature is somewhat elevated but there is no regional lymphadenopathy. A sticky hyperptyalism is present. Slide examination shows a few cocci with fusiform bacilli and spirochaetes. A 72-hour blood agar culture shows, with the exception of a few staphylococcus albus, almost a pure culture of streptococcus viridans. No fungi are found by culture on Sabouraud's medium. Treatment with 1:500 metaphen locally and saturated aqueous solution of copper sulphate cautery of the hypertrophic tissue brought fair results.

In another somewhat similar case there were large, painful superficially eroded, non-infiltrated patches over the mucous membrane. These were covered with a whitish pseudo-membrane which

* Gins claims some motile funiforms are degenerate spirillae.

was easily wiped away to leave a red and inflamed substratum. The surrounding mucosa was bright red. No fusiform bacilli, spirochaetes, or fungi were demonstrated. Blood agar culture showed staphylococcus aureus and streptococcus viridans. Treatment with metaphen as above described achieved a regression of symptoms.

Interesting though these reported cases may be, it is our impression that the author has insufficient evidence to support a contention that the streptococci are the etiological agents. It has already been pointed out earlier in this paper that the streptococci have been isolated from virtually 100 per cent of the normal mouths. It is not too much to suspect that the primary cause of such a disease syndrome as described by Woodburne might be something other than a normally occuring mouth inhabitant.

Belding and Belding (15) reported the discovery of what they describe as an extremely pleomorphic organism from the mouth. They do not mention the culture media employed or the technique of isolation. From the photomicrographs accompanying their article it appears to us that they were dealing with a mixed culture. There is insufficient evidence to support the assertion that more species pleomorphism would account for all the variations in form depicted by their photomicrographs. Until more evidence is forthcoming their "extraordinarily pleomorphic" organism must be considered as several organisms growing in one culture.

The bacteria isolated from 400 tonsils were the subject of a

study by Cobe (16). At the time of operation the tonsils were placed in sterile gauze which had been previously dampened with saline. This was in turn wrapped in waxed paper and promptly placed in the refrigerator. Not over four hours later the tonsils were dropped in boiling water to coagulate the surface, then cut with a sterile scalpel, and the cut surface streaked in sunburst fashion upon a blood agar plate. The indications for operation in 10 per cent of the cases was the recession of an acute tonsillitis. In the remaining 90 per cent chronic inflammation was present. Table four shows the results.

Some 200 of the operations were performed during the spring months and the remainder during the autumn. A definite seasonal variation in incidence of the bacteria found was reported. Friedlaender's bacillus was found to occur five times more frequently in the spring than in the fall and the diphtheroids, gram-positive cocci and Bacillus proteus twice as often. Bacillus influenzae occurred 80 per cent more often in the spring than in the fall. The only one which showed a significant increase in autumnal incidence was Micrococcus catarrhalis which increased by 100 per cent at this time. These findings may be of interest if one speculates on the factors influencing the health of the child at these two periods. The background flora is practically the same as has been found in adults by other workers but this variation in incidence is of interest. It may be that following exposure to the sun's rays, increased outdoor life, and perhaps in-

gestion of fresh fruits and vegetables over the period of the summer months, the child's immunological mechanism is built up to such a point that resistance to the organisms inhibits their growth in the tonsils. An investigation of the antigenic titre of the blood of these patients against strains of the organisms isolated from their tonsils would be of considerable interest. Determination of any seasonal variation here would be of considerable significance in developing a rationale for spring coryza prophylaxis.

Smith (17) took a bit of a Vincent's infection membrane, macerated it in sterile horse serum, and injected it immediately into the groin of two guinea pigs. Two weeks later large foul smelling abscesses developed. When pus from these lesions was injected intra-tracheally in rabbits, four developed lung abscesses, three lung gangrene, two pneumonia, and one bronchiectasis. The pus from the lung abscesses and the gangrenous cases showed cocci, vibrio, small fusiform, but no large fusiform or spirochaetes. While this work is of interest, it does not appear to us that complete proof of the direct etiological role of these organisms has been established. The so-called fusospirochaetes have been considered by many investigators to be saprophytic and devoid of a primarily pathogenic role. It would have added much to the proof had Smith injected a bit of the membrane which had been rendered sterile. It is entirely conceivable that some of

the necrotizing metabolic products in the membrane might produce sufficient damage locally to permit already extant bacteria to grow. In a personal communication Dr. Smith States: "This point has been controlled in my original work. If the necrotic material is sterilized by pasteurization no infection results. The same test was applied to the experimental infection in rabbit's lungs. When the pyorrhea material was pasteurized, no infection occurred in the animal's lungs."

Cline reports the isolation of several new strains of anaerobic bacteria some of which have an apparent definite relationship to the higher forms. This work is of considerable interest since it points the way for such additional work which may be of value. Technical difficulties have heretofore prevented any intensive work on anaerobic forms but the work of Varney (18), Fortner (19), and others has provided methods which are effective. The discovery of forms which have a relatively long latent incubational period is of considerable interest since it may indicate the explanation for failure to establish a bacterial etiology for some of the commoner diseases. It is conceivable that after failure to cultivate an organism in the customary period of incubation, the investigator is tempted to declare the disease under question a virus disease. It is possible that some of the commoner diseases heretofore attributed to the viruses may be caused by these anaerobic forms which possess such extended latent incubational periods that they have escaped discovery and isolation. Illustrative of this is the Bacterium melaninogenicum.

Pleomorphic anaerobic leptothrices were found in considerable number by Cline. It is noteworthy that by virtue of their pleomorphism they could readily be mistaken for bacillary forms in a purely morphologic study. Again the consideration arises that

these organisms may play a pathogenic role.

In his work with the anaerobic forms, Gins found liver-peptone-water and liver gelatine the most satisfactory media. In employing these two he could ascertain the more differential characteristics of each of the species under investigation. Gelatine liquefactive powers, gas formation, odor production, colony formation, and blackening of the pieces of liver to which considerable significance was attached, could all be observed. Table 5 summarizes his findings. In a study of this table one is impressed by the pleomorphic tendencies of the bacteria. The purity of his cultures is somewhat open to question and confirmation is awaited. The findings are nevertheless of interest because they indicate that there is relatively unexplored field for bacteriological research. Animal experimentation to determine any pathogenic properties of the described species would be of value.

Some mycologic aspects of the oral flora

Before a full evaluation of the role played by higher bacterial and fungal forms in the production of disease can be achieved, gaps in our knowledge about these forms must be filled. Prior to Pasteur's time the fungi were considered to be exceedingly important factors in disease production. With the publication of researches on anthrax (Pasteur), cholera (Koch), diphtheria(Klebs), and others, interest in mycology was transferred to bacteriology. It has only been of recent years that the fungi have emerged as

possible potent factors in the production of chronic illness. Tremendous taxonomic difficulties face the new worker in mycology. Henrici (30), Castellani (21), and Dodge (23), to name a few mycologists, all offer their own classifications of the fungi. These differ in so many points that confusion instead of clarification arises from a study of their works. Difficulties in this field are increased by the weeks and months of time which must pass before certain diagnostic colony formations develop. Until a standard nomenclature is devised for the mycologists there will be much difficulty in pursuing a systematic orderly study of the higher bacteria. Consequently the mycotic etiology of many diseases will remain obscure and the treatment empiric.

MacFarlan (33) stated that "leptothrix, streptothrix, yeasts, and molds are often found in the normal mouth." He observes that in overwhelming yeast infections of the mouth there is a recession of the lower bacterial forms. Many more pathogenic higher bacteria are said to exist than are commonly recognized. Iodides have been found useful in the treatment of most mycotic infections and, awaiting discovery of more specific therapeutic measures, he recommends the routine use of these compounds in cases of demonstrable mycotic etiology. C

Chart 1 is taken from Castellani's (24) report on the tonsillomycoses. It serves as a useful indication of the type of fungus which may occasionally be found in the mouth. That these organisms may have a definite pathogenic property is shown

by his descriptions of several different mycotic infections of the tonsils. They are summarized here according to his scheme:

1.-Acute tonsillomycosis (follicular)
Symptoms: Greyish white spots about the follicle opening. Sore throat and discomfort in swallowing. Maybe a fever. May spread to uvula and soft palate. Heals spontaneously in 1-3 weeks.
Diagnosis: Usually by the discovery on a slide of large numbers of yeast cells with but few or no spirochaetes.
Treatment: Glycerin or 5% phenol locally. Potassium iodide systemically.

2.-Diphtheria-like tonsillomycosis
Symptoms: Sudden onset, sore throat, dysphagia, prostration, fever 102-103, often a regional lymphadenitis. Cream-white pseudomembranous patches which bleed on removal.
Diagnosis: Few spirochaetes and absence of diphtheria organisms.
Treatment: Chlorine gargles with 10 per cent tincture of iodine locally.

3.-Tonsillactinomycosis (rare)
Symptoms: Slowly developing abscess with unilateral lymphadenitis. Then ulceration with sinus formation and expulsion of typical sulfur granules.
Diagnosis: Made on the finding of the sulfur granules.
Treatment: Castellani recommends iodides but Myers (38) indicates that thymol systemically and locally is practically a specific.

4.-Tonsillopseudomycosis
This is the same as the tonsillactinomycosis except that sulfur granules are missing. Treatment and diagnosis is essentially the same.

5.-Tonsillomycosis fusca
Symptoms: Acute to subacute onset with formation of brownish or yellowish spots about the tonsils.
Diagnosis: Brownish pseudomembrane. Large mycelium which give cerebriform colonies on glucose agar. This is usually caused by a trichosporon.
Treatment: Potassium iodide systemically.
Remarks: This is found in India, South Africa, Italy, and Balkans. It is very refractory to treatment.

6.-Tonsillomycosis spinulosa
Etiology: Oidium, trichosporon, or hemisporon
Symptoms: Grey to brownish spicules emerging from the crypts. Maybe a slight sore throat is the only complaint.
Diagnosis: Made on the finding of fungi in smears from the tonsillar spicules.
Treatment: None or iodides.

Relatively rare though these condition may be, they may have a greater incidence than is suspected. Without an examination of smears by a competent observer, they might well be confused with other mouth conditions.

As in any superficial lesion, secondary bacterial or mycotic infection may occur in the mouth. Brain (25) reports a case of a 49 year old white female with a unilateral superficial erosion of the tongue of 14 months duration. Examination showed symmetrical, irregular sunken patches with no visible papillae. The adjacent tissue was white and mottled. In the lesion itself was found a yeast-like organism which showed budding and the cultural characteristics of a monilia. Wrist lesions simulating those found in lichen ruber planus were found. The Wassermann reaction was negative. A diagnosis of primary lichen ruber planus with secondary monilia infection was made. The monilia responded to massive doses of potassium iodide. Sir Aldo Castellani confirmed the diagnosis and commented that 50-60 per cent of normal persons harbor monilias in their mouths. While this case report is interesting, one wonders what the effect would have been had the therapy been

directed toward the primary disease. It is possible that had this been cleared the monilial infection might have been simultaneously cured.

Norris (36) examined by smear and culture 210 sputum specimens from tuberculosis suspects. In these he found yeasts present in 8.5 per cent, and of this same group 45 per cent contained tubercle bacilli. The sputums which manifested only yeasts were characterized by slightly less greenishness and purulence than the tuberculous sputum. Symptoms of this type of pulmonary mycosis were: cough, slight fever, loss of strength, weakness, rales, fullness in the lung bases, and a leukopenic tendency with relative lymphocytosis. It is readily apparent that the differentiation of tuberculosis and mycosis would be difficult. One is constrained to wonder how many patients are confined to sanitaria for tuberculosis when they are in reality suffering from pulmonary mycosis. Using Sabouraud's medium Norris found that in 48 hours small white, glistening, colonies with pearly raised borders and irregular dases would develop. In media with low pH he found that mycelium formation was stimulated while budding was more prone to occur in the more alkaline media. The organisms usually fermented dextrose and maltose and produced acid in lactose and saccharose broths. They varies from 4 to 10 micro in size, were ovoid, refractile, and greenish in color. Gentian violet in solutions of 1: 10,000 killed them.

Injection of a pure culture into an experimental rabbit produced a leucocytosis with the lymphocytes predominating. Death resulted from inanition and the rabbit manifested little evidence of toxicity. Agglutinins, precipitins, and opsonins were demonstrable in the rabbit serum. Agglutination in a titre of over 1:80 was considered distinctly significant.

One of Woodburne's (14) patients manifested greyish patches on the oral mucosa. The erosions had an erythematous base and an inflammatory halo. In some of the regions they were quite hypertrophic. The lesions were chronic and in exacerbations were accompanied by a regional lymphadenitis. Leukoplakia was closely simulated by these lesions. When a bit of the pseudomembrane covering the lesions was digested in 20 per cent NaOH it showed a considerable mycelial growth. On Sabauroud's medium almost a pure culture of Monilia albicans were obtained. Lugol's solution given systemically twice a day and silver nitrate coutery locally every other day brought about a gradual regression of the symptoms.

Weidmann (27) has described some very helpful laboratory procedures in the establishment of diagnoses of mycotic infections. Microcurretage of the suspected lesions with a Pasteur pipette and subsequent examination of the scrapings, with culture on Sabauraud's or glucose agar, will frequently establish the presence of this type of infection.

Warthin (26) and Tunnicliff and Jackson (29) have reported the discovery of two rather interesting organisms from the tonsillar

crypts. In Warthin's report the described specimen was obtained in only 2 of 50,000 tonsils examined post-surgically. In these instances the tonsillar crypts contained spherical bodies 30-300 micra in diameter which had hyaline walls and were closely packed with unripe spores. Sporangia with ripe spores were likewise observed. No hyphae were noted. Warthin concluded that while the organisms resembled Rhinosporidium seeberi in many respects it was not. In Tunnicliff's report, rosette and test-tube brush-like forms were obtained on culture. These simulated the original material obtained from actinomyces-like tonsillar granules. Ovoid and irregular appearing bodies and filaments were observed. Appearing to originate from and situated about these were noted bacillary, filamentous, and the previously described forms. The organisms were recovered in pure culture from an abscess produced in a rabbit by subcutaneous injection of a suspension of these organisms. The vibriothrix was reported as gram-negative, non-acid-fast, and a slowly developing anaerobe.

COMMENT AND SUMMARY

It is apparent that the oral bacterial flora, pathogens in systemic as well as local disease, is being more closely and critically studied. New facts pertinent to already recognized species are summarized here and descriptions of new organisms of both bacterial and higher forms are included.

Table 1.

"Per Cent Incidence of Bacteria in Mouths of Mothers and Their Newborn Children" According to Witkowski

Bacterial Species	Per Cent Occurrences in Children Shortly After Birth	Per Cent Occurrences in the Mothers
white staphylococci	72	100
hemolytic staphylococci	---	44
streptococci	22	90
hemolytic streptococci	---	14
pneumococci	---	60
bacillus lacticus	48	---
colon bacilli	72	64
Friedlaender's bacilli	---	34
micrococcus catarrhalis	---	12
corynebacterium	---	38
yeast	---	54
sarcinae	---	18
proteus	---	4
sterile	2	---

Table 2.

"Per Cent Incidence of Bacteria in Difference Clinical Types of Mouths"
"according to Wohlfeil and Schultz."

Condition of teeth.	number of cases	hem strep	hem staph	#	enterococcus	#	strep lact.
FROM THROATS							
Good	195	24.1	7.7	194	14.9	186	39.0
Average	232	21.2	4.9	212	15.6	191	27.7
Poor	86	18.6	2.3	84	33.3	80	31.3
Total	503	21.8	5.6	490	18.3	457	26.1
FROM TEETH							
Good	194	0.5	2.1	196	20.8	185	29.2
Average	215	1.3	0.4	213	23.0	193	24.4
Poor	88	1.1	0.0	88	34.9	81	38.4
Total	505	1.0	1.0	491	24.8	459	28.0

TABLE 3

Principal bacterial forms encountered in 50 normal mouths

Type of organisms	Per Cent Incidence				Per Cent in Which Predominating			
	L.L.A.	L.P.M.	S.D.	W.D.	L.L.A.	L.P.M.	S.D.	W.D.
Gram ± bacillus	82	86	80	90	54	30	28	36
Gram - bacillus	80	86	86	78	20	20	12	10
Gram + staphylococcus	70	78	54	60	14	28	16	18
Vibrio	56	64	48	40	0	0	4	0
Gram + diplococcus	42	34	72	64	0	2	22	8
Gram + Coccobacillus	30	24	30	56	0	4	6	12
Gram - staphylococcus	22	20	14	18	0	4	0	4
Gram + streptococcus	22	26	16	22	0	0	0	0
Gram - diplococcus	18	22	36	24	0	0	4	0
Gram - coccobacillus	8	14	30	16	0	2	2	0

L.L.A. = Lower Lingual anterior region.

L.P.M. = Lower posterior molar.

S.D. = Stenson's Duct.

W.D. = Wharton's Duct.

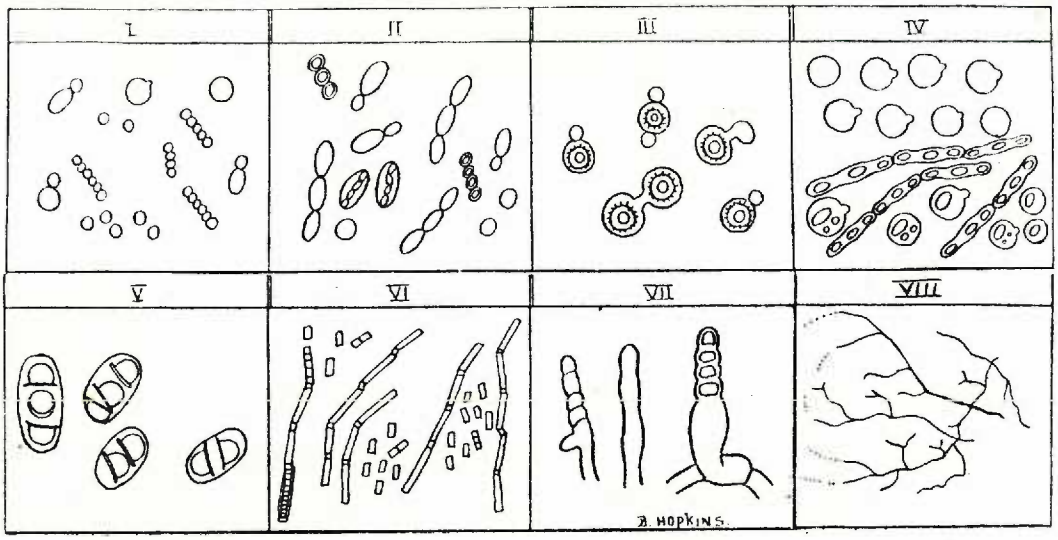
Table 4.

"Per Cent Incidence of Bacteria in Inner Portions of Tonsils Removed at Operation"
According to Cobe.

Organisms	Per Cent Occurrence	Comments
staph. aureus	68.5	
staph. albus	22.75	
staph. citreus	2.00	
pneumococcus	58.25	bile soluble g plus dip.
strep. hem.	30.75	
strep. non-hem.	14.5	
strep. viridans	7.5	
B influenzae	23.25	no special attempts to raise.
M. catarrhalis	26.75	
B. mucosus caps.	20.75	
diptheroids	11.25	
g plus cocci	6.75	
B. proteus	2.25	

CHART ONE
from Castellani

FIG. 1.—Principal types of fungi found in Tonsillomycoses.



I Cryptococcus.
II. Saccharomyces.
III. Debaryomyces.
IV. Monilia.
V. Willia.
VI. Oidium (*sensu* Piñoy).
VII. Hemispora.
VIII. Nocardia (Streptothrix).

Hansen, 1904; *Endomyces* Rees, 1870. Fungi of the

References

1. Brooks, James, W.: A regional morphologic study of normal oral flora. Jour. Dent. Research. (to be published)

2. Witkowski, Ruth: Aerobe mundhohlenflora bei mutter und kind. Zentralbl. fur Bakt. 133: 334, Feb. 18, 1935.

3. Fleming, Alexander and MacLeon, Ian H.: Occurrence of influenza bacilli in mouths of normal people. Brit. Jour. Exper. Path. 11: 127, April, 1930.

4. Wohlfeil, T. und Schulz, H.: Vergleichende Untersuchungen uber die aerobe mikrobenflora bei Zahngesunden and Zahnkranken. Zentralbl. f. Bakt. (Abt. 1) 128: 219, April 28, 1933.

5. Gundel, M. and Linden, H.: Die flora der mundhohle Gesunder und thre Bedeutung für die pathogenesis der erkrankungen der atmungsorgane. Zentralbl. f. Bakt. (Abt. 1) 121: 349, July 13, 1931.

6. Smith, David T.: Oral spirochaetes. Williams and Wilkins Co. Phil. 1933.

7. Arnold, Lloyd and Steard, Carroll W.: Recent developments in the study of oral bacterial flora. Am. Jour. Digest. Dis. and Nutrit. 2: 275, July 1935.

8. Pettit, Joseph A.: Lesions of the oral cavity. Northwest Med. 33: 38, Feb. 1934.

9. Cins, H. A.: Die nichtsporenden Anaerobier der mundhole und der Zahne. Zentralbl. f. Bakt. 132: 129, Aug. 7, 1934.

10. Levington, Herbert: Review of views on Vincent's infection. Internatl Jour. of Orthodontia. 21: 1171, Dec. 1934.

11. Broughton-Alcock, W.: A case of invasion of the intact buccal mucous membrane by spirochaetes. Proc. Roy. Soc. Med. 23: 1017, May, 1930.

12. Wolbach, Dept. S. and Howe, Percy R.: Intercellular substances in experimental scorbutus. Arch. Path. and Lab. Med. 1: 1, Jan., 1926.

13. Tunnicliff, Ruth: Relation of spiral organisms to the rough colony of B. fusiformis. Jour. Bacteriology. 55: 290, Sept.-Oct., 1925.

14. Woodburne, Arthur R.: Some interesting and unusual lesions of the oral mucous membrane. Jour. Mich. State. Med. Soc. 34: 356, June, 1935.

15. Belding, P. H. and Belding, L. J.: Oral Flora. Jour. Am. Dent. Assoc. 24: 963, June 1937.

16. Cobe, Herbert Marshall: Incidence of bacteria in 400 tonsil cultures. Jour. Infect. Dis. 46: 293, May, 1930.

17. Smith, David T.: Fusospirochaetal disease of the lungs produced with cultures from Vincent's angina. Jour. Infect. Dis. 46: 303, 1930.

18. Varney, P. L.: A simple method for culturing anaerobes by means of phosphorous. Jour. Lab. and Clin. Med. 11: 193, 1925.

19. Kuhn, Joseph: Ein einfaches plattenverfahren zur Züchtung stronger anaerobier (anaerobe bazillen = filtrierbare anaerobe bakterien = spirochaete pallida) Zent. f. Bakt. 108: 155, 1928.

20. Henrici, Arthur T.: Molds, yeasts, and actinomycetes. John Wiley and Sons, New York, 1930.

21. Castellani, Sir Aldo. Arch. Derm. and Syph. Oct., Nov., Dec., 1927 and Jan., Feb., and Mar., 1928.

22. Dodge, Carrol William: Medical mycology. C. V. Mosby Company, St. Louis, 1935.

23. MacFarlan, Douglass: The clinical aspect of oral mycology. The Laryngoscope. 44: 29, Jan., 1934.

24. Castellani, Sr Aldo: Tonsillomycoses: a brief general account. Practitioner. 126: 67, June, 1930.

25. Brun, R. T.: Lichen planus with lesions of the tongue and buccal mucous membrane and secondary monilia infection. Proc. Roy. Soc. Med. 23: 1043, Feb. 20, 1930.

26. Morris, Jack C.: Further observation on the pathogenic yeasts. Jour. Med. Assoc. of Ga. 22: 250, July, 1933.

27. Weidman, Fred D.: Laboratory Diagnosis of the oral mycoses. Laryngoscope. 44: 41, Jan., 1934.

28. Worthin, Alfred Scott: Occurrence of unidentified sporangia in the tonsil crypts. Arch. Path. 12: 33, July, 1931.

29. Tunnicliff, R. and Jackson, L.: Vibriothrix tonsillaris N. Sp: the organism of actinomyces-like tonsillar granules. Jour. of Infect. Dis. 46: 12, 1930.

30. Howitt, Beatrice: Cultural and serological reactions of lacto-bacilli from the mouth. Jour. of Infect. Dis. 46: 351, May 1930.

31. Lichtenberg, R. H., Werner, Marie, and Enock, Esther: Pathogenicity of the fusiform bacillus and spirillum of Plaut-Vincent. Jour. Am. Med. Assn. 100: 707, Mar. 11, 1933.

32. Myers, H. H., "The fungicidal activity of Certain Volatile Oils & Stereoptens" J. A. H. A. 84: 1935, June 27, 1935.

Table 5
Differential Characteristics of the
Gins Strains of Oral Non-sporulating Anaerobes.

Bacterial strain	Morphology	Gas	odor	gelatine liquefaction	tissue darkening	hemolysis	methemoglobin	Colony Appearance	Pleomorphism	Source	Incubation time before growth	Remarks
Gi. I	Large diplococcus	-	-	-	-			2mm. terraced shiny	Marked with age of colony	Membrane about root tip		
Gi.XXII	Streptobacilli on plates. Streptococcoid & filamentous forms in fluid media.	-	-			-	-	Blood agar shows punctate colonies with small superimposed ons.	Only moderate.	Case of Vincent's angina	"few days" on plate. Blood agar 48 hours.	Resembles organism of Kreilorsky-Ivankeritch.
Gi.XXVI	Filamentous inter twining bacilli. Parellel chains of streptobacilli. Usually pseudomycellias not 7.5 micron	-	-	-	-	-	-	Colorless, small, with raised center & flat fibrous-like periphery.	Marked tendency for formation of degenerating & involutional forms.	Case of Vincent's Angina.	7 days on Forkner plate	Virtually same as Gi.XXII.
Gi. X	Streptobacilli at inner margin of colony. Some clumping	+++	+ in liver bouillon	+	+ also renders them pultaceous			Stellate with fading radial tongues.	Slight	Case of Vincent's angina.		Pultaceousness of tissue in liver bouillon not observed with other anaerobes.
Gi.XVIII	Network of filaments and bacillary forms. Red granules in young forms with Loeffler methylene blue. Absent in older ones.	-	-	-	-	-	-	Rough, punctate, small.	Marked. Often looped forms to resemble large spirochaete	Granuloma of tooth.	24 hours slight. several days for typical colonies.	Periphery of colonies show rough web of pleomorphic bacilli. Stains readily with Giemsa stain.
GiXIX	Resembles B. fusiformis Delicate vacuolizations very regular banded appearance in single colonies.	-	-	-	-			Grey-green small, moist shiny, regular in appearance	Marked. Some organisms show fusiform swelling at center.	Case of paradentosis. Also from fistule from subphrenic abcess.	8 days	Indole reaction at 8 weeks. Very poorly stained with Giemsa. Older forms appear granular, irregular and shrunken.
Gi.XX	Often over 25 micra long. Wound & tangled. A leptothrix	-	-	-	-	-	-	Small, punctate, thin, some large and milky with root like protusions coalescing.	Marked.	Case of paradentosis	5 days.	

Gi.XX	Often over 25 micra long. Wound & tangled. A leptothrix	-	-	-	-	-	-	Small, punctate, thin, some large and milky with root like protusions coalescing.	Marked.	Case of paradentosis	5 days.	
.XXIII	Leptothrix, large wound forms with radiating flagella-like projections. Some short fusiform bacilli. Some arranged in strips of 3. gram .	-	-	-	-			Stellate with developing head in center	Moderate. Inner colony shows filaments. Indented forms at periphery.	Case of paradentosis.	24 hrs. for hazy growth. "Several days" for colonies.	When in strips of 3, the end organism due to rotation on their own long axis showed an indented outline. If these were not bent, would be indistinguishable from B. fusiformis.
Gi.XV	Leptothrix. Rods with abruptly pointed or rounded, never rectangular, ends. Resembles the "Milzbrand bazillen".	-	-	-	-	+		Fine transparent grey white filagree which in 6 days covers the whole plate.	Chain formation in center of colony. Filaments at periphery. Some coccoid forms.	Case of paradentosis & from Vincent's angina.	2 days.	In liver bouillon growth is as tufts on the tissue bits. Greater number of bacillary forms distinguishes from Gi.XXIII. Indented forms present in this strain also.
Gi.XIV	After involution of center of colony. Short bipolarly stained bacilli c granular inclusion bodies. At periphery are spirillar forms c filaments.	no growth	-	no growth	-			1mm. Smooth edge, colorless, shiny, moist. Grow into the media.	Marked. Delicate spirals often lie beside filaments twice as thick as the bacillary forms.	Pyorrhea alveolaris and a carious tooth.	5 days.	With Giemsa stain bacilli are transparent with sharp blue borders. Spiral forms stain more intensely than the filamentous.
Gi.XXVII	Very large & broad filaments at periphery. Turns on own long axis often. A leptothrix.	-	-	-	-			Friable, root-like projections which are taken up c difficulty. Older, there are filamentous tufts at periphery.	Moderate.	Carious teeth.	1-2 days.	Resembles GiXXIII in having indented forms.
Gi.IX	Leptothrix. Width of forms 1/2-½ of a RBC. Stains polychromatically. Filaments of variable thickness in older colonies. Rounded	++	+	-	-	-		Small, sparse.	Marked.	Case of pyorrhea alveolaris.	11 days.	Gins believes this identical with Miller's Bacillus buccalis maximus.
Spirillum buccale (Fortner)	Spirillar forms of vigorous motility. Older Zettnow stain shows peritrichate flagellae-a diagnostic finding.	-	-	-	-	-	-	Delicate hazy, carpet like & eventually covers whole plate	Young forms have high steep curves which flatten c age & reduce their motility. 3 wk. cultures show filamentous forms.	Tonsil.	"several days"-- in 2 days if warm 20% blood plates used.	Long filaments with fine steep coils are fragmented flagellae. Liver-milk coagulated in 2 days. Old forms resemble B. fusiformis. Gins says the motile fusiform bacilli were this organism.

	Short bipolarly stained bacilli & granular inclusion bodies. At periphery are spirillar forms & filaments.	no growth		no growth		loss, shiny, moist. Grow into the media.	lie beside filaments twice as thick as the bacillary forms.	and 9 carious teeth.		Spiral forms stain more intensely than the filamentous.
Gi.XXVII	Very large & broad filaments at periphery. Turns on own long axis often. A leptothrix.	-	-	-	-	Friable, root-like projections which are taken up & difficulty. Older, there are filamentous tufts at periphery.	Moderate.	Carious teeth.	1-2 days.	Resembles GiXXIII in having indented forms.
Gi.IX	Leptothrix. Width of forms 1/3-$\frac{1}{2}$ of a RBC. Stains polychromatically. Filaments of variable thickness in older colonies. Rounded	+	+	-	-	Small, sparse.	Marked.	Case of pyorrhea alveolaris.	11 days.	Gins believes this identical with Miller's Bacillus buccalis maximus.
Spirillum buccale (Fortner)	Spirillar forms of vigorous motility. Older Zettnow stain shows peritrichate flagellae-a diagnostic finding.	-	-	-	-	Delicate hazy, carpet like & eventually covers whole plate	Young forms have high steep curves which flatten & age & reduce their motility. 3 wk. cultures show filamentous forms.	Tonsil.	"several days"-- in 2 days if warm 20% blood plates used.	Long filaments with fine steep coils are fragmented flagellae. Liver-milk coagulated in 2 days. Old forms resemble B. fusiformis. Gins says the motile fusiform bacilli were this organism.
Gi.XVII	Small slightly bent rod. Sometimes flattened spirillar forms which are several terminally joined vibrios. Also has peritrichate flagellae.	no growth		no growth		Very fine film. At periphery has pine-tree configuration & regular side branches. Individual colonies. Have stellate arrangement.	Absent.	Tonsil.	3-4 days.	Probably closely akin to Spirillum buccale of Fortner.
XXV.	Slender bacillus of variable length. Smears show the bacteria arranged to simulate runic inscriptions. Faint staining & usual stains.	no growth	no growth	no growth	+	Minute, round, yellowish, perceptible sheen, definite halo, grow into the media.	Runic and spirillar forms present.	Case of paradentosis.	5 days.	

Part II

REGIONAL STUDY OF BACTERIA IN CLINICALLY NORMAL MOUTHS

Since Miller (1) there has been but little study devoted to the strictly morphologic aspects of the oral flora, either in the clinically normal or in the diseased mouth. Smith (2) recently has redirected attention to the important role that spirochetal forms seem to play in the pathogenesis of some oral lesions. Despite the exhaustiveness of his review, a clear concept of their pathogenicity escapes us. In Vincent's infections the fusiform bacilli and spirochetes are reputedly the chief malefactors. Recently (10, 15, 20) doubt has been expressed about their sole responsibility and somewhat more attention has been devoted to the accompanying organisms. Culture methods have been employed in the study of the other bacteria. Time does not permit the practicing dentist--or physician--to use these methods routinely. He must rely on the examination of stained smears. Neither a careful evaluation of smear diagnoses nor a morphologic tabulation of bacteria apparently normal to certain regions of the mouth is available. As a preliminary to a study of Vincent's infection, this was deemed necessary. Since bacteriological methods are employed by the dentist almost exclusively for the diagnosis of Vincent's infections, the organisms supposedly causative of this disease have received the greater attention in our study.

The literature on oral bacteria is abundant. Topley and Wilson (3) indicate that the normal flora comprises chiefly micrococci, staphylococci, streptococci, gram positive and gram negative bacilli, and

spirochetes. They do not make a more specific listing. Gundel and Linden (4) ascertained that the four pneumococci groups, the influenza bacillus, and the Friedlander bacillus were frequently found in healthy mouths. They determined, moreover, that these organisms showed a seasonal variance in incidence, being present in greater numbers during the winter and spring months. Miller (1) reported the isolation of more than one hundred different organisms from the mouth. Lloyd and Stuart (5) doubt the value of a study of the oral flora. They assert that the difficulty in finding a healthy mouth, together with the multiplicity and variability of the organisms encountered makes the establishment of bacterial norms a hopeless task. Various workers report the almost constant presence of the alpha hemolytic streptococci in the clinically normal mouth. The pyogenic cocci, together with micrococci such as the Neisseria, are said to be consistently present.

Lord (6) reported the presence in the normal of actinomycetoid organisms which on guinea pig inoculation produced lesions histologically identical with those of actinomycosis. In five instances the results were culturally confirmed while eleven cases (the five included) showed organisms morphologically identical with the actinomyces. In 1936 this worker with Truet (7) stated that no difference in morphology and staining reactions could be detected between the actinomycetoid organisms of the mouth and the demonstrably pathogenic actinomyces of the Wolff-Israel type. It was further noted that the cultural differences on continued development in vitro

vanished--except for the obligate anaerobiosis of the Wolff-Israel organism. Naeslund's reported fulfillment of Koch's criteria for pathogenesis was not confirmed but was, nevertheless, regarded as sufficient justification for considering the actinomycetoid organism as a part of the normal oral flora. Hoffman (8) reports the isolation of a leptospira and implies that it may reach an incidence as high as 40 per cent. This organism has not been obtained in pure culture and its pathogenicity is undetermined. Smith found this bacterium (Leptospira trimerodonta, Hoffman, 1921) in only one of 100 mouths he examined. No description of the mouth or region from which the smear was taken was mentioned. Seuat (9) describes an organism, Leptothrix racemosa, which he says is frequently encountered. The identity of this is doubted by Smith. The fusiform bacilli, which are reputed to play a symbiotic role in the etiology of Vincent's infections, are reported present in various numbers. Smith is of the opinion that these organisms are present in all mouths but that any local disease condition permits their multiplication. Different workers have attempted subdivision of this group of bacilli according to their morphologic, cultural, and antigenic structure. A recent work by Smith (10) classifies them on a morphological basis into three groups. Hine (11) has reported the isolation of 23 strains of the fusiform bacilli and suggests that the extent to which they depress the pH of sugar broth cultures could be used as a basis of classification. Tunnicliff (12) claims isolation in pure culture of the fusiform bacillus and advances the phyothesis that it is but

a stage in the life cycle of the oral spirillum. More recently Beust (13) has asserted that the descriptions of the bacilli have been based on inadequately stained preparations and that as a result the morphologic, cytologic, cultural, and biochemical characteristics are confused. He does not believe that as yet the fusiform bacillus has been cultivated. Curtsell (14) simultaneously reports successful isolation of this bacillus. Her pure culture when injected into two rats produced a Vincent-like lesion in one. Varney (21) after an intensive study ascertained that morphology alone would permit rough classification of the fusiform bacilli into four groups. The pleomorphism of the strains and their varied appearance on different culture media makes such a distinction insecure. He advocates, consequently, a classification on a basis of antigenic structure. At the present time considerable confusion about the oral spirochetes exists. Smith's monograph follows the classification of Noguchi (15) and refers to seven treponemata: T. buccale, T. vincenti, T. macrodentium, T. microdentium, T. mucosum, T. skoliodontum, and T. orthodonium. In addition to these, Miller's Spirillum sputigenum is said to be omnipresent. Noguchi (15) states that the T. vincenti is present in small numbers in the normal mouth. Greenberg and Greenberg (17) reported 78 per cent of 109 smears from healthy gingivae as free from spirochetes. Only one of the 50 spirochete positive smears was said to contain more than a very few of these organisms. Fifteen of the 50 showed fusiform bacilli and spirochetes also. Other authors report varying incidences up to 50

per cent. There seems to be general agreement that some of the spirochetes are present in about 30 per cent of normal mouths but the individual identity of these spirochetes is not given.

The vibrios have of late attracted scant interest although Miller devoted considerable attention to them. Their pathogenicity and incidence in the clinically normal mouth has, so far as we know, not been determined. An etiological role in the production of infectious mononucleosis has been suggested but not proved (22). Vibrio viridans is believed to play a symbiotic role with other organisms in the etiology of Vincent's infections (10, 16).

The reaction of the mouth organisms to Gram's stain has attracted interest of some investigators. Picard's results, reported by Missle (18) indicate that during infancy, the lingual mucosa is dominated by gram positive organisms but with approaching age the gram negatives supervene. In oral diseases the greatest number of bacteria is again gram positive--61 of 64 smears from diseased mouths showing gram positive organisms predominating. He arrived as the conclusion, on what appears to us to be an insufficient number of cases, that the type of diet did not influence the ratio of gram positive to gram negative organisms.

It is apparent that, if one is to rely on a stained preparation in the diagnosis of oral lesions, a knowledge of the bacterial flora peculiar to the clinically normal mouth is essential. While numerous investigations of the bacterial inhabitants of the tonsillar crypts, the alveolar sockets, and the pharynx have been made, we have been

unable to discover a report on the purely morphologic aspects of the bacteria found in various regions of the mouth. As a preliminary to a study of Vincent's infection this was considered to be of value.

METHOD

Fifty medical students and nurses permitted us to take smears from their mouths which were first pronounced clinically normal by a dentist. A normal mouth was arbitrarily defined as one in which there appeared no evidence of (a) gum recession, (b) pericementitis, (c) gingivitis, (d) active caries, or (e) ulceration. Four sites for obtaining the smears were selected. These were (a) the lingual surface of the lower incisors, (b) the lingual surface of the lowermost, posterior molar, (c) the entrance of Stenson's duct, and (d) the entrance of Wharton's duct. The first two sites were selected on the assumption that they would be representative locales, the latter two to ascertain the salivary influence on the flora. A 2 mm. wire loop, previously flamed to incandescence, was stroked in the longitudinal tooth axis or about the duct orifices. A drop of diluting fluid (formalin 10 drops, glacial acetic acid 20 drops, water to 25 c.c.) was placed on a clean new slide and the loop then used to spread the fluid over a circular area whose diameter was not over 1 cm. This was air dried, fixed with heat, and stained by our modification of the Gram stain. The stain technic employed was as follows: 5 per cent $NaHCO_3$ and 1 per cent genetian violet in proportions of 4:15 were freshly mixed and allowed to act for 7 minutes. After washing in water, Gram's iodine was applied for 3 minutes. Washing and decolorization was performed as usual. The counterstain, 1 per cent carbol-fuchsin, was applied for 40 seconds. Under this routine the gram-positive organisms assumed a deep reddish purple stain while the gram-negative were pink. This stain routine was found to show the morphology of the spirochetes to best advantage and yet preserve much of the differential value of the gram stain.

A quantitative tabulation of the stained organisms was considered impossible of achievement owing to the uncertain factors of: (1) salivary dilution, (2) variation in area covered by the smear on the slide, and (3) variation in area from which the smear was taken. A qualitative evaluation was done, assuming that regardless of the factors introducing error into the quantitative study, the proportions of the micro-organisms to each other would remain relatively constant. Accordingly the organism occurring oftenest in ten fields was rated four plus, the next most frequent a three plus. If a considerable number were present but yet outnumbered by two other types, a two plus rating was given. Anything else present was considered one plus.

The bacteria were classified on a basis of gram-reaction and morphology. The basis for differentiating the spirochaetes was taken from Smith (2) and Noguchi (15). It is given here: 1.--T. buccalis. A wide organism with irregular sinuous coils, variable in length, deeply staining, and blunt ended. 2.--T. vincenti. Half the thickness of T. buccalis, irregular spirals usually 3 to 6 in number, tapering ends, readily stained. 3.--T. macrodentium. About the size of T. vincenti but with tighter regular coils, short, sharp ends, and slighter staining intensity. 4.--T. microdentium. Thinner than the above but with tight-set regular spirals, tapered ends, and diminished staining intensity. 5.--T. mucosum and T. pallidum. These two organisms, the former reputedly a symbiote in the production of Vincent's infections and the latter found in syphilitic lesions of the mouth, are morphologically indistinguishable from T. microdentium. They were included under the latter name, its being understood that T. microdentium might signify either that organism or T. pallidum or T. mucosum. The serological tests for syphilis were negative in all the experimental subjects in this series so it is very unlikely that T. pallidum was included--even if it could be stained, a feat reportedly impossible of achievement with the reagents used. 6.--T. skoliodontum. A small organism with irregular coils, thin body, and tapering ends, poorly staining and measuring over-all 2 to 4 microns. 7.--T. orthodontum. Morphologically indistinguishable from the skoliodontum and included under that name. 8.--Leptospira trimerodonta. A delicate, thin organism with tight-set spirals and the hooked ends typical of the leptospirae.

RESULTS

The results are summarized in the accompanying tables. That spirochetes together with fusiform bacilli are found in 45 out of 49 normal mouths is in itself suggestive. Of the 4 mouths not demonstrating these 2 organisms, 3 harbored fusiform bacilli. In at least 10 of the 45 fusiform bacilli and spirochete positive mouths, smears were indistinguishable from those obtained in cases of acute Vincent's infections. It was noted that when desquamated epithelial cells were encountered the bacteria usually occupied the periphery of the cell with but a few in the paranuclear region. This suggests that the intercellular cement substance affords better growth facilities for the bacteria than the cell itself. The portal of entry for many

pathogens may thus be speculatively explained.

Table I shows the incidence of the bacteria found in smears from each of the 4 locations in the mouth. It is to be noted that, while a certain bacterium may be present in a high per cent of cases, it does not necessarily dominate the filed. The gram positive bacilli are exceptions to this, as they tend to predominate. Gram positive staphylococci are present in greater frequence and greater numbers in the region of the posterior inferior molar than at any other site. This may be of some help in explaining the fact that this region is the commonest site of post-extractive infectious complication.

A significant fact is revealed in Table II. The frequency of occurrence of the spirochetal forms about the teeth and their relative scarcity about the orifices of the ducts suggests one of two things: either the teeth afford some growth promoting factor, or the saliva exerts an inhibitory effect. It is apparent from the facts here that the diagnosis of a Vincent's infection chiefly on the finding of fusiform bacilli and spirochetes ought to be made with caution, especially if the lesion is in the paradental region. Table III substantiates this assertion. Here it will be observed that the incidence of fusiform bacilli and spirochetes is quite high in the region of the teeth but very low near the ducts. It is also noteworthy that the presence of spirochetes unaccompanied by fusiform bacilli, or the reverse, is rare. Tunnicliff's theory of the identity of these two organisms offers an interesting tentative explanation.

Of particular interest to us was the high incidence of acti-

nomycetoid forms and of fusiform bacilli, as shown in Table IV. Castellani (23) and MacFarlan (24) have described mouth infections due to pathogenic forms of a morphology similar to the organisms we observed. It would seem plausible that these organisms may be potentially pathogenic and that they, under conditions of lowered local resistance, may play a primary role in the production of a clinical syndrome readily mistaken for Vincent's infection. It is our opinion that the part the higher bacterial forms play in the production of mouth pathology has not been sufficiently investigated. The approach to this must be through a study of the organisms present in the clinically normal mouth. Table IV indicates the high incidence in this variety of mouth.

DISCUSSION

From the standpoint of oral diseases, particularly Vincent's infection, it would appear that the smear examination is open to misinterpretation. It is apparent that, if 10 out of 45 non-diseased mouths show the bacterial picture of an acute Vincent's infection, smears in such instances could readily lead one to consider himself dealing with a Vincent's infection when actually the lesion was of another etiology and entirely masked by the bacterial picture of Vincent's infection--a picture we conclude is normal to 20 per cent of non-diseased mouths. Clinical signs must be held of more importance than a bacterial examination and, even in the presence of typically positive slides, if the clinical appearance is not complete the diagnosis of a Vincent's infection is insecure.

The importance of fungi in mouth diseases ought not to be overlooked. In the examination of the 200 slides here reported, we were impressed by the frequency of occurrence of the higher bacterial forms. Because of their morphologic similarity to the actinomyces, all were recorded under the heading of actinomycetoid organisms. Any relatively long filamentous form which showed almost rectangular ends or evidence of branching was included in this category. The ease of misteking a higher for a lower bacterial form in routine smear examinations is obvious. A fragment of a mycelium, were one not particular to note the usual abruptness of the ends, could be mistaken for a fusiform bacillus.

The validity of a morphologic classification of the various treponemata must be questioned. As yet not all the oral treponemata have been obtained in pure culture. Consequently, limits of specific morphology have not been defined, nor may one exclude the possibility, for example, of T. skoliodontum being a post-divisional or variant phase of T. vincenti. The mere fulfillment of morphologic criteria does not constitute identification. The various treponemata as yet unstudied in pure culture may have forms which coincide, under certain conditions, with those attributed to the more thoroughly studied species. One is probably justified in concluding, if a number of organisms in a preparation answer the description, that one of the better studied species is present. The identification of individual organisms is as yet impossible.

More recent studies of anaerobic mouth bacteria may contribute

much to a better understanding of oral diseases. Gins (25) has discovered new anaerobic forms which may play an important role in the production of disease. The newly discovered Bacterium melaninogenicum (25 and 26) may have considerable importance not only in oral, but also in systemic disease.

We feel, as a result of this incomplete study, that the diagnosis of Vincent's infection from a smear preparation alone ought not to be made. If a positive slide is found, it should be considered of little significance, since we have found that some 20 per cent of clinically normal mouths show fusiform bacilli and spirochetes in numbers sufficient to warrant a diagnosis of acute Vincent's infection. The negative slide in the presence of positive clinical signs, however, should cause one to consider a diagnosis of Vincent's infection with skepticism.

SUMMARY

An investigation and morphological tabulation of the bacteria found in 4 locations in 50 clinically normal mouths is reported. From the high incidence of fusiform bacilli and spirochetes, it is concluded that the diagnosis of Vincent's infection chiefly on the appearance of a smear preparation is of questionable accuracy. Attention is directed to the frequent occurrence of higher bacterial forms.

REFERENCES TO LITERATURE

1. Miller, W. D., Micro-organisms of the mouth. (Philadelphia: S. S. White Dental Mfg. Co., 1891)

2. Smith, D. T. The oral spirochetes. (Baltimore: Williams & Wilkins Co., 1932.)

3. Topley, W. W. C. and Wilson, Gr. S., The principles of bacteriology and immunity, (New York: William Wood & Co., 1928), p. 1257.

4. Gundel, M. and Linden, H., Zentralbl. f. Bakt., Orig., 121: 349, 1931.

5. Arnold, L. and Stuart, C. W. Am. J. Digest. Dis. and Nutrit. 2:275, 1935

6. Lord, F. T., Boston Med. and Surg. J., 163: 82, 1910.

7. Lord, F. T. and Treuet, L. D., J. Infect. Dis., 58: 115, 1936.

8. Hoffmann, E., Zentralbl. f. Bakt., Orig., 86: 135, 1921.

9. Beust, T. B., J. A. D. A., 14: 389, 1927.

10. Smith, D. T., J. A. D. A., 23: 1136, 1936.

11. Hino, M. K., J. D. Res., 15: 169, 1935.

12. Tunnicliff, R., J. Infect. Dis., 33: 147, 1923.

13. Beust, T. B., J. D. Res., 15: 168, 1935.

14. Cartmell, R., J. D. Res., 15: 187, 1935.

15. Noguchi. H., The Spirochaetes...chpt. 36 in Jordan and Falk, Newer knowledge of bacteriology and immunology, (Chicago: University of Chicago Press, 1928)

16. Wesson, J. H., J. A. D. A., 23: 1483, 1936.

17. Greenberg, S. and Greenberg, F., D. Cosmos, 71: 364, 1929.

18. Miesle, Zentralbl. f. Bakt., Orig., 82: 346, 1934.

19. Noguchi, H., J. Exp. Med., 15: 61, 1912.

20. Merritt, A. M., J. A. M. A., 25: 2027, 1936.

21. Varney, P. L., J. Bact. 13: 275, 1927.

22. Corbum, L. W., Hunt, H. D., and Smith, D. T., J. Clins. Invest., 7:504, 1929.

23. Castellani, A., Practitioner, 128: 67, 1930.

24. MacFarlan, D., Laryngoscope, 44: 29, 1934.

25. Gins, H. A., Zentralbl. f. Bakt., 132: 129, 1934.

26. Liebetruth, E., Ztschr. f. Hyg. u. Infectionskr., 116: 611, 1935.

TABLE I

Principal bacterial forms encountered in 50 normal mouths

Type of organisms	Per Cent Incidence				Per Cent in which predominating			
	L.L.A.	L.P.M.	S.D.	W.D.	L.L.A.	L.P.M.	S.D.	W.D.
Gram + bacillus	82	86	80	90	54	30	28	36
Gram - bacillus	80	86	86	78	20	30	12	10
Gram + staphylococcus	70	78	54	60	14	28	16	18
Vibrio	56	64	48	40	0	0	4	0
Gram + diplococcus	42	54	72	64	0	2	32	8
Gram + Coccobacillus	38	24	30	56	0	4	6	12
Gram - staphylococcus	22	20	14	18	0	4	0	4
Gram + streptococcus	22	26	16	22	0	0	0	0
Gram - diplococcus	18	22	36	24	0	0	4	0
Gram - Coccobacillus	8	14	30	16	0	2	2	0

L.L.A. = Lower Lingual anterior region.

L.P.M. = Lower posterior molar.

S.D. = Stenson's Duct.

W.D. = Wharton's Duct.

TABLE XI

Regional incidence of the various Treponemata in 50 normal mouths

Organisms	Per Cent Incidence			
	L.L.A.	L.P.M.	S.D.	M.D.
Spirillum	32	20	8	2
T. vincenti	56	46	8	1
T. macrodentium	36	22	8	0
T. microdentium	20	20	2	0
T. buccale	16	16	2	2
T. skoliodontum	10	12	2	4
Leptospira trimerodonta	0	2	0	0

TABLE III

Co-incidence of spirochetes and fusiform bacilli in 50 normal mouths

Organisms	Per cent Incidence			
	L.L.A.	L.P.M.	S.D.	W.D.
Spirochetes and fusiforms	88	78	16	14
Spirochetes without fusiforms . . .	2	0	10	4
Fusiforms without spirochetes . . .	0	2	0	0

TABLE IV

Regional distribution of fusiform bacilli and actinomycetoid forms in 50 normal mouths

Organisms	Per Cent Incidence			
	L.L.A.	L.P.M.	S.D.	W.D.
Fusiform bacillus	88	80	16	14
Actinomycetoids	61	64	35	41

Part Three

"Mycotic Gingivitis: a case report."

Castellani, Dodge, Henrici and other mycologists have described lesions of the oral mucous membranes resulting from monilia infection. Thrush in children is perhaps the best known of oral diseases of mycologic origin. Various workers have called attention to the importance of considering higher bacterial forms in the etiology of stubborn, treatment resistant mouth lesions. A case in point is reported here.

CASE REPORT

A sixty year old white female entered the Out-patient Service of the University of Oregon Medical School complaining of burning and moderate tenderness of the gingivae. Three years prior to entrance she had suffered a cerebral vascular accident which left her with a partial hemiplegia. Shortly after this, she noted a series of vesicles appearing over both aspects of the gingivae. These at first were productive of discomfort only by virtue of their physical dimensions. Within a period of two weeks, the vesicles proceeded thru pustulation to ulceration at which time the lesions became more extensive, spreading to involve the frenulum linguae, the uvula, and the soft palate as well as the gingival tissue. At the time of the attack, the patient suffered from general malaise of only moderate severity. She consulted a dentist who cauterized the lesions with a substance the patient states was chromic acid. This treatment accomplished a partial regression of the symptoms. After approximately two months the lesions gradually

receded leaving a residual hyperaesthesia of the gingivae which had never been entirely subsided. She had suffered "several" attacks similiar to this initial one, but they had all been of lessened severity. There was no history of asthma, hay fever, urticaria, or other allergic complaints.

On examination, the gingivae were found to be rolled, bright red in color with a translucent appearance, moderately firm, smooth, shiny, and easily induced to bleed. No calculus or gross evidence of pus pockets was discernible. Over the posterior portion of the palate there was a greyish, reticulated, easily detached membrane with a dusky bluish periphery. There was no odor and no regional lymphadenitis.

With the exception of a slight relative leucopenia the blood and urine studies were negative. The Wasserman reaction was negative. The basal metabolic rate was slightly lowered. No marked reaction was detected to the common allergens. Blood agar cultures, in addition to the usual oral flora, yielded a fungus whose characteristics are detailed below.

The patient's blood serum agglutinated a saline suspension of the organism to a titre of 1:1024 in one instance but this was not confirmed on susequent agglutination tests. A phenolized saline suspension of the organism taken from a Sabouraud's slant growth at 96 hours and adjusted to a turbidity of 3 on the $BaSO_4$ nephelometric scale gave an erythematous wheal 1.5 cm in diameter at

48 hours when 0.05c.c. was injected intra-cutaneously. This wheal remained visible for six months after the injection, becoming slightly pigmented.

On the assumption that the organism was a monilia and probably the etiologic agent in this instance, the patient was given 0.5 grams of thymol in capsules b.i.d. and this supplemented with aqua thymolis mouth washes t.i.d. After two weeks of this treatment, the patient complained of symptoms attributed to a mild gastritis and the thymol was discontinued. No improvement was noted subjectively or objectively. Feeling that an allergy might play a role, the patient was given 0.50 cc of the phenolized test suspension subcutaneously three times a week. This was gradually increased over a period of two months time to 0.5 cc. At the end of this period, the gums had lost their shiny inflamed appearance. They did not bleed on pressure. The burning sensation had dimished and the patient stated that her mouth felt better than it had for several years. At this time, she suddenly left the clinic and further study was impossible.

Laboratory Study of the Organism

No particular attempt was made to identify the organism. It was found after an examination of the literature that each mycologist had a different concept of nomenclature and that little agreement existed as to the characteristics of even the general. An objective listing of the growth characteristics was then considered to be probably of more value. When a generally accepted mycological

nomenclature is devised it may be that the cultural reactions listed here will be found to have been sufficiently extensive to permit identification.

Plate Reactions

Table one illustrates the colonial and microscopic characteristics. It will be noted that a 20% sucrose infusion agar is the only medium which gives a grossly detectable variation from the usual appearance of the colony.

Table two gives the composition of the experimental media described by number in Table one.

Table three gives the biochemical reactions of the different sugars. The organism apparently utilizes glucose and is capable of splitting it off from the disaccharides, maltose and sucrose. The mechanism of its utilization is not clear. It is logical to assume that were the molecular dissolution to proceed by the commonly accepted routes, the abstraction of the CO_2 moiety from a hexose, leaving a pentose, from a pentose a tetrose, and so on, that these intermediate substances would be just as readily utilized by the organism as the glucose. This, however, does not occur. The organism does not grow on media enriched by any of these substances. Insufficient data is at hand to warrant speculation on this process, which is probably of academic interest only. Plate one illustrates the microscopic appearance of the organism.

Summary

A case is reported by mycologic gingivitis, which apparently

responded to desensitization. The cultural and biochemical characteristics of the isolated fungus are tabulated.

TABLE ONE

Plate Growth Charneteristics of Oral Fungus Isolated
From case of Chronic Gingivitis

Medium	Inc. Time	Colour of Colony	Size of Colony	Edge of Colony	Consistency of Colony	Satellite Colony	Done of Colony	Size of Organism	Hyphae	Budding	Comment
2% Blood agar	24m	Creamy	2mm	Regular	Putty-like	None	Shiny, convex	10-20U	Few	Many	Good growth
Experimental Media #1	48m	White	2mm	Regular	"	None	Shiny, convex	10-30u	Few	Few	Poor growth
20% Sucrose nest infusion Agar	24m	White	3mm	Irregular	"	None	Wrinkled	1525u	Many	Many	Excellent growth
Infustion Agar #50mg le30 4	24m	Rust	2mm	Regular	Soft	None	Smooth	10-20u	Few	Few	Poor growth
Experimental Media #2	No Growth										
Plain agar ē 10% lactose	6 days	white	2mm	Regular	Powdery	None	Powdery	10-20u	Spicular	slight	Poor growth
Experimental Media #3	72 hr.	Creamy	0.5mm	Regular	Soft	None	Convex	15-25u	Few	Few	Poor Growth
Experimental Media #4	No Growth										
Sucrose Agar anaerobically	No Growth										

TABLE TWO

Composition of Experimental Media

Experimental Media Group Number	Base	Substances Added	Quantity Added
Number One	Meat Infusion Agar	Asparegin	10 mg per plate
		Staphylococcous filtrate	0.5cc per plate
		Malic acid	10 mg + 20 mg per plate
		Aspartic acid	10 mg + 20 mg per plate
		" "	20 mg " " " " "
		Rosolic acid	10 and 20 mg per plate
Number Two	Meat Extract Agar	Clove Oil	2 minims per plate
	"	Thynial	10 mg 1 plate
Number Three	Plain agar	Sucrose	1 + 2 cc of 40% solution
		Maltrose	1 + 2 cc of 40% "
		Lactose	2 cc of 40 % "
		Glucose	1 + 2 cc of 40% "
Number Four	Plairi agar	Gentian violet	1 + 2 minims of 2% soluti
	"	Methyleru blue	1 + 2 " " 1% "
	"	Nothing	
	Meat Infusion	Rhamnose	10 mg
		Urea	20 mg
		Raffinose	10 mg
		Inulin	10 mg
		Sorbital	10 mg
		Anyl alcohol	3 minims per plate
		Methanol	3 " " "
		Glactose	1 cc of 10% solution
		Lactic Acid	3 minims per plate

TABLE THREE

Biochemical reations of Fungus Isolated from Case of Chronic Gingivitis

Medium	24 hrs.	72 hrs.	Comment
Glucose broth	ACT	ACT	Turbid growth
Maltose broth	A	ACT	Turbid ē surface pellicle
Lactose broth	Growth slight	Growth slight	NoAC. growth in tube butt.
Sucrose broth	Growth slight	A	Turbid uniformly
Mannilt broth	Growth slight	Heavy growth	No AC heavy sediment
Plain broth	Slight growth	Slight growth	Moderate turbedity