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## Anaerobic streptococci : a history, classification, habitat and pathogenic activities, culture methods, and clinical aspects

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The Anaerobic Streptococci:  
History, Classification, Habitat and Pathogenic  
Activities, Culture Methods, and Clinical Aspects.

by  
Lawrence J. Gridley

Presented To  
The College of Medicine  
of the  
University of Nebraska  
Omaha, Nebraska  
1941

## PREFACE

The coming of the knowledge of the existence of microorganisms as agents of disease and the swift advances in this field which followed the works of Pasteur and Lister brought to Medicine a new life and initiated great steps forward.

As Medicine continues to widen its field of knowledge and institute new and more efficacious therapeutic measures, we see, still playing an important role in this advance, the science of Bacteriology.

One phase of this science is that which deals with the anaerobic microorganisms, and a small phase of this phase is that which has to do with the anaerobic streptococci.

Perhaps it is because of this relative size of the subject to the science of Bacteriology as a whole as well as to the whole field of Medicine, that little accord has been paid in the text books and in the literature in general to this study. In looking through the theses submitted in the past by Seniors of this institution I was not confronted with one which had to deal with this

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subject.

In this paper I have attempted a thorough review of the literature on the subject of the anaerobic streptococci, their history, classification, differentiation, habitat and pathogenic activities, culture methods, and the relation of these subjects in general to clinical medicine.

I wish to express my sincere appreciation for the suggestion of the subject, for advice as to how it might best be covered, for his translation of the section on history of the theses of the French worker, Prevot, and for the pleasant manner in which he offered his cooperation to doctor Millard F. Gunderson of the College of Medicine of the University of Nebraska. Also to John M. Slack for his helpful advice, my thanks.

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## INTRODUCTION

In the case of many scientific facts, a concept may be proven to be true and yet remain generally unknown for a remarkably long period of time. This is true in regard to the anaerobic streptococci and their relation as an etiologic factor to many diseases. The pathogenicity of these organisms has for a considerable time been recognized by many, but it is still denied by some, and its importance not apparent to a great number of men in the field of practical medicine.

Anaerobic streptococci, while formerly considered rare as pathogens, are assuming an increasingly prominent position as a known etiologic factor in certain inflammatory conditions of the human body.

It has been only since the very latter part of the last century that any work has been reported in regard to these important agents of disease. The early work along these lines was done by a group of French workers, (see section on History). This was followed by a series of reports in Germany, and it has not been until quite recently that this work has been taken up in this country.

The outstanding reason for the lag in the acquisition of knowledge concerning this subject has been

the lack of simple, practical, suitable methods for the anaerobic cultivation of micro-organisms. Also as a factor in the slowness of uptake of such a study may have been the fact that so little was known about the relation of these organisms to disease that even if their etiologic importance had been recognized, still there were no known differences in the therapeutic management of such conditions, if they did exist, from that of other similar infectious processes, so inspiration to further work on the basis of reward in the form of better response to a more intelligent and a more specific form of treatment was lacking.

It is my purpose in this review of the literature to show that there is an important relationship between the anaerobic streptococci, as etiologic factors, and many disease processes, some of them common and of relatively great importance, and some of them rare and of rather less importance, but each of them no doubt rather important to whatever person who may be afflicted with one of them. It is also my purpose to show that there is a rather large number of strains or species of these organisms, that they may be roughly classified and differentiated, that they are path-

ogenic to animals and to man, that there are simple and practical methods of cultivating and studying these organisms, and that after having cultivated and determined the anaerobic nature of the organisms found in a particular lesion, that there are in many instances valuable specific methods of treating such a lesion and that these methods vary from and are more efficacious than the previously standard methods of treating such lesions.

I wish to emphasize the importance of making anaerobic studies of all specimens from lesions requiring bacteriological study as an aid to diagnosis.



## HISTORY

In 1884, Rosenbach had thoroughly described under the name, *Micrococcus fetidus*, an anaerobic micrococcus found in dental caries. (1)

In 1886, Lemaistre (2), seems to have been the originator of the true concept of anaerobiosis within the genus, *Streptococcus*, with respect "*Streptococcus plicatilis*", the streptococcus of Perleche as found in chronic ulceration of the lip in children. He obtained pure cultures on a medium made of gel of *Fucua Crispus*. On this medium he developed numerous well separated colonies, some of which, containing only diplococci, reproduced upon inoculation into broth beautiful specimens of streptococci. Lemaistre believed that this organism was an anaerobe and that that was the reason for its occurrence at the junction of the lips.

The French school is credited with emphasizing not only the anaerobic streptococci, but also the entire group of anaerobic cocci because it was the first group to describe these organisms and to recognize important pathogenic significance.

Veillon (3), in 1893, found in the putrid pus of Bartholinitis, in a fatal Ludwig's Angina, and in a perinephritic abscess, a putrefactive and gangrene-

producing streptococcus occurring in short chains. He described this organism under the name of Micrococcus fetidus. This erroneous classification was due, most likely, to the fact that he was unable to recognize the true generic position of the organism because its streptococcal morphology was not at all times evident. Later, however, when questioning a streptococcus found by the German workers, Kronig and Menge, and classified by them as Micrococcus fetidus, Veillon and his assistants determined the streptococcal characteristics of this organism. Experimentally, according to Prevot, (4), this organism produces only hot abscesses, but Veillon offered it as a cause of putrid suppuration.

In a series of theses, Veillon's students have described this organism, its frequency, habitat, and the pathological conditions in which it may be found.

Rist (5), in 1898, tells of finding Micrococcus fetidus in particular as well as other pyogenic and gangrene-producing organisms in a large number of purulent infections of the ear occurring at the point of attachment of the auricle. This work proposes:

1. The precise description of Micrococcus fetidus;
2. To call attention to a special feature presented

by a growth of certain anaerobic streptococci in agar deeps, which feature follows the work of R. Muller (6), and later that of Graf and Wittneben (7), and which is called for the sake of convenience by the title, "the phenomenon of alternate zones", because they consist of alternate zones of sterility and of growth in the upper parts of the Veillon tubes.

Halle, (8), also in 1898, showed the presence of *Micrococcus fetidus* in the normal and in the infected vagina, in genital infections, in cases of retained placenta, in pus of Bartholinitis, etc. He foresaw its pathogenic nature by the experimental production of infections, abscesses, or gangrene at times fatal to the animals, using pure or mixed cultures. Halle compared *Micrococcus* with the anaerobic streptococcus of Kronig and Menge and thought that they were perhaps the same.

In 1898, Guillemot, (9), recovered *Micrococcus fetidus* from thirty per-cent of purulent processes, putrefactive and gangrenous, of the respiratory tract. This organism associated with *B. Ramosus*, with *Staphylococci*, or with facultative streptococci, reproduced pulmonary gangrene in animals. The strains of *Micrococcus fetidus* which Guillemot found in pulmonary

gangrene differ from the strains found by Veillon, Halle, and Rist in other putrid suppurations and especially does it bear less resemblance to a streptococcus than the latter.

In 1899, Jules Cottet, (10), found six strains of *Micrococcus fetidus* in urinary abscesses. This emphasizes the importance of the role of anaerobes in periurethral gangrene.

Thus we see a picture of the important work done in beginning the establishment of a new knowledge, a knowledge of *Micrococcus fetidus*, the first species of anaerobic streptococcus up to the time of this work.

A second species was isolated in 1901 by Lewkowitz, (11), and this species was well differentiated since it is neither putrefactive nor gangrene-producing. Lewkowitz, in the laboratory of a Professor Grancher, during the course of investigations on the microbic flora of the mouth of nurslings, found among other habitual anaerobes, two cocci, one of which is a very small streptococcus and which he called "*Streptococcus anaerobius micros*", but which for the sake of conformity with the binomial system of nomenclature, has later been renamed, *Streptococcus*

micros (Lewkowicz). Thus in dropping out one part of the original name the part, anaerobius, has been dropped in order to avoid confusion with the species of Kronig of similar name.

In 1902, Jeannin (12), put in relief the role of anaerobic streptococci in putrid puerperal infections.

In a first series of forty-one cases of post-partum putrid puerperal infections, Jeannin found eight strains of *Micrococcus fetidus* and five strains of other anaerobic streptococci. On a second series of twenty-one cases, he found six strains of *Micrococcus fetidus* and nine strains of other anaerobic streptococci.

On a third series of nineteen cases (putrid post-partum retained placenta), he found three strains of *Micrococcus fetidus* and six strains of other anaerobic streptococci. Within this series there was a mortality of 35% due in part to gaseous septicemia caused by *Micrococcus fetidus* and *Streptococcus anaerobius*. In three cases of gaseous septicemia with retained fetus he found two strains of *Micrococcus fetidus*.

As one of the anaerobes found in uterine gangrene

he again mentions *Micrococcus fetidus* and says that it forms gas in all the infections where it is found. In the benign puerperal infections and also in the normal state he finds that the vulva and the vagina but chiefly the vagina, conceal anaerobic streptococci, either or both *Micrococcus fetidus* and *Streptococcus anaerobus*.

Later in a case of hydatidiform mole which terminated with hemorrhage, he found an anaerobic streptococcus in the blood. Prevot, (4), states that this case is to his knowledge the first case known in which anaerobic streptococci were to be found in the blood and was earlier than the cases claimed by the German workers to give them priority in this regard. Jeannin concludes that the causative organisms of puerperal infection are the same as in other putrid infections and that among the most frequent species should be considered *Micrococcus fetidus* and the other anaerobic streptococci.

It is of passing interest to note the first complete work on the genus, *Streptococcus*, in which there is mention of the anaerobic species of this genus. This work is a monograph on the streptococci by Le Gros, (13). Le Gros emphasizes that Pasteur

discovered the streptococci and they were so named by Billroth in 1874 and not by Rosenbach as it is so frequently stated. He reminds us that the genus was considered as an aerobic bacterium, and that Lemaistre discovered the anaerobic nature of some of its species. In conclusion this author describes the *Streptococcus micros* (Lewkowicz) and concludes as to the impossibility of classifying but the possibility of grouping according to their agreements, the facultative species of streptococci because of their plurality.

Prevot in his thesis, (4), states, "At the beginning of this epoch, we see not more in France than some notes either of some workers recognizing the presence of anaerobic streptococci in some new cases or attempting to determine their precise role".

Rut, (14), in 1902, in a note on the bacteriology of seven cases of suppurative salpingitis noted three cases due to anaerobes. In one of these three, appeared an anaerobic streptococcus identical with that of Kronig and Menge - (see Classification).

Also in 1902, Gilbert and his pupils, (15), presented a series of notes on the presence of anaerobic streptococci in the bile ducts. Gilbert and Lipman found some strict anaerobes including an anaer-

obic streptococcus in the normal flora of the extra-hepatic bile ducts. Gilbert and Lipman, (16), found anaerobic streptococci three times in the bacteriology of cholecystitis. One of these was *Micrococcus fetidus*. In concluding their study, these authors (17), determined the frequency of these organisms in cases of cholecystitis to be twenty-five per-cent.

Lipman and Foisy, (18), found *Streptococcus anaerobius* in an acute osteomyelitis of the femur "a porte d'entree pulmonaire".

Gourand, (19), reported anaerobic streptococci in a puerperal infection and in a pulmonary gangrene.

Guillemot, Halle, and Rist, (20), presented new evidence on the question of anaerobic streptococci in two papers. One paper on history and technique emphasized the importance of the anaerobes, among them *Micrococcus fetidus*, which they found in five out of thirteen cases of putrid pleurisies, and of which they gave a new description.

In their second paper these three authors discussed their work in attempting to reproduce experimentally with the use of pus, the putrid pleurisies. Some of the conclusions in regard to *Micrococcus fetidus* at which these men arrived are especially



worthy of note:

"These strictly anaerobic bacteria do not constitute a special flora in the putrid pleurisies. They are there in all the suppurations of a putrid or gangrenous nature, having as their origin the digestive tract and its annexes, the respiratory apparatus, or the genito-urinary system."

"Many of these organisms may be found as saprophytes on the mucosa of such natural cavities."

Prevot in his thesis stated that precise information on the true role of *Micrococcus fetidus* in pure culture and the nature of the lesions which it causes is lacking.

Rist in a review in 1905, (21), summarizes the accepted views on the pathogenic anaerobes from suppurative gangrenes, in which the position of these micrococci is important, in particular *Micrococcus fetidus* and *Streptococcus anaerobius* of Kronig and Menge. Special emphasis is placed on their role in pleural and pneumonic gangrene and in infections of the female generative tract.

Prevot's conception of the role of the anaerobes in gangrenous and necrotic processes is briefly portrayed in the following few words. The anaerobes live

in the natural cavities. They move into a neighboring lesion, multiply rapidly, and invade the process, thus proclaiming their proper pathogenic role, resulting in gangrene of the lungs, genitalia, appendix, biliary tract, mouth, ear, etc.

The finish of the initial work on the question of the anaerobic streptococci was marked by the work of Jeannin (22), in 1907. In a series of seven cases of puerperal infection each of which terminated in recovery, he found anaerobes in five cases. Of these anaerobes, three times they were found to be *Streptococcus anaerobius* and twice *Micrococcus fetidus*.

Parallel to the work of the French group was that of the German school but the beginning and ending of this work respectively were later than in the case of the former.

Kronig and Menge were the pioneers of the German school.

In 1895, Kronig (23), found a large number of strains of streptococci. These he attempted to classify into two species only. The first of these, no doubt, corresponded to a mixture of putrefactive and gas-forming forming species and not any one well defined species. For this reason it has been difficult,

if not impossible, for the species studied and defined by the French workers to be either compared with or identified with the Streptococcus of Kronig or any of the German strains. Regarding the second species of Kronig, it differs from the first only in the fact that the length of the cells is greater and "the cells are often gathered in a heap". It may be that this second species is the same as the French Micrococcus fetidus, but it is impossible to say for sure.

Menge (24), studied the flora of the vulva and vagina, and recovered anaerobic streptococci.

Kronig and Menge combined resources and published a treatise on the bacteriology of the female generative tract, (25), in which they claim to have found anaerobic streptococci twelve times in the vaginal secretions of sick and normal females.

Natvig in 1905, (26), recovered six strains of anaerobic streptococci from infected lochia, all of which, like Kronig and Menge's strains, produced gas in culture media. Natvig suggested that the entire group of anaerobic streptococci should be called "Streptococcus anaerobius (Kronig)". He failed to produce lesions in animals.

14.

bacteriologic study of one hundred ninety-two fall-

15.

opian tubes which had been removed surgically; he found five strains of anaerobic streptococci.

Prevot, (34), in 1925, reported and classified thirteen strains of strictly anaerobic streptococci. He added one strain to those previously described and called it, "Streptococcus intermedius". He reported this organism as being a medium sized diplococcus, (0.6 micron in diameter), which produced neither gas nor odor in cultures but coagulated milk. Suspensions of live organisms injected alone into guinea pigs and mice produced only small abscesses, but if injected with a filtered toxin of *Vibrion septique*, they produced widespread, crepitant lesions.

Harris and Brown, (35), in 1928, reported the isolation of seventeen strains of anaerobic streptococci from the vaginas of thirty normal women in the puerperium. These same men, (36), the following year reported on a study of fifty-seven strains of anaerobic streptococci from infected lochia of one hundred sixty-eight women in the puerperium. They suggested a classification based on sugar reactions and reactions on blood media.

Taylor, (37), in 1929, reported on eleven strains of strictly anaerobic streptococci obtained from

infections of frontal sinuses, pleurisy, pulmonary gangrene, and dental sepsis. Because of the variations observed in sugar fermentation reactions, he concluded that a classification upon such a basis would be of no value.

Soule and Brown, ( 38 ), in 1932, obtained anaerobic streptococci from cervical cultures in forty per-cent of women examined.

In the past ten years the amount of work being done on the question of the anaerobic streptococci has increased to a noticeable extent. Prominent have been the works of Meleny, Brewer, Reicher, Christopher, Alexander, et al on the subject of chronic, undermining, burrowing ulcer of the skin. There has also been a good deal of work done in the past ten years on the subject of anaerobic streptococci in regard to puerperal infection.

## BACTERIOLOGY

### A. General Considerations

The following is an attempt at a brief treatment of the bacteriological features of the anaerobic streptococci in general. More specific and detailed information concerning any of the strains or species in particular may be had from the section on Classification and Differentiation where Ford's, (39), description of the different species is to be found.

#### I. Colonial and Morphological Characteristics.

Four chief colony types have been described by Colebrook and Hare, (40). These are described later in the section on Classification. Horse blood agar and glucose agar with ten per-cent horse serum have been used by these men as the best media for the study of these organisms.

Growth in fluid nutrient medium -- optimum pH for growth in fluid media is 6.8 to 7.0. Colebrook and Hare have found the most satisfactory medium to be a simple tryptic digest of ox liver. Poor growth occurred on digested heart broth and little or no growth in peptone broth. In the digested ox liver and in meat medium the anaerobic streptococci produce a very character-

istic and unpleasant odor due to the formation of skatol. The majority of the type A strains form gas in this medium. (see Classification). The addition of some ascitic fluid or serum favored the growth of especially type B strains.

## II. Metabolic Activities.

1. Gas formation -- many of the anaerobic strains of streptococci, unlike their aerobic brethren, produce gas in abundance when grown in mince meat medium or in the liver digest medium spoken of above. The type A organisms in particular show this power.

2. Carbohydrate fermentation -- Colebrook and Hare found that about half their strains did not ferment any of the test sugars. Of the remaining half, none showed any clear grouping which could be correlated with their colonial characters.

3. Decomposition of sulphur compounds -- sodium thiosulphate (1%) in fresh state was blackened by all of the strains of Colebrook and Hare. Sodium sulphate (1%) was not blackened by any of the strains. Sodium sulphite (1%) was blackened by only a few of the type A strains.

Sodium persulphate (0.5%) was not blackened by any of the strains, but did not allow abundant growth of the organisms. Cystin was blackened by nearly all strains.

4. Indol formation -- very poor or no growth on 1% peptone broth. Only very few positive results on liver digest medium incubated under vaseline seal for several days and then testing with Bohme's reagent after shaking with ether.

5. Clotting of milk -- only scanty growth obtained. Clotting occurred with one strain tested by Colebrook and Hare.

6. Proteolytic fermentation -- the above authors added gelatin to liver digest broth (to make 15% gelatin), and the organisms were incubated under vaseline seal for six days at thirty-seven degrees C. When the cultures were subsequently cooled in the refrigerator, fifteen out of seventeen solidified. Only two strains digested the gelatin.

7. Heat resistance -- when heated for half an hour at fifty-eight to sixty degrees C., eight strains grown in liver digest broth under vaseline



were all killed.

### III. Serological Differentiation

Colebrook and Hare, (40), prepared agglutinating sera in rabbits for eight strains of anaerobic streptococci. Attempts were made to determine the antigenic relations of the homologues and twelve other strains of organisms. Simple agglutination and agglutinin absorption tests were employed. There was experienced considerable difficulty in obtaining suspensions sufficiently stable for testing by the water bath method. Six of the eight sera tested by the thick suspension method with one to ten dilution of the sera showed agglutination only with the homologous anaerobic streptococci. The antigenic analysis of these anaerobic streptococci presents a problem more difficult to solve even than that of the *S. pyogenes* group because of the difficulty in obtaining growth in artificial media.

Colebrook and Hare's types A and B were shown by them to be serologically distinct.

Biochemical and serological tests are not yet sufficiently reliable to serve as a really good method of differentiation of the anaerobic

streptococci. It is probable that they are a number of serologically distinct types. The two commoner types of anaerobic streptococci of Colebrook and Hare, (types A and B), correspond broadly but probably not exactly with *Micrococcus fetidus* of Veillon, (*S. putrificus* of Schottmuller), and *Streptococcus anaerobius micros* of Lewkowicz.

Of clinical interest is the finding of Colebrook and Hare, (40), that anaerobic streptococci, unlike *S. pyogenes*, *S. viridans*, and other aerobic varieties, are unable to multiply freely in human blood or serum, but when the alkali reserve of the serum is reduced or the antitryptic power of the serum neutralized, they are then able to grow quite rapidly. It is suggested that conditions of local acidosis may play an important part in favoring infections by these organisms.

## B. Classification and Differentiation

Numerous attempts have been made to classify the streptococci which occur in diverse lesions and to establish some differential criteria by means of which they can be separated from the numerous non-pathogenic saprophytic strains. According to Ford, (39), no classification has been found entirely satisfactory, and for the present we must regard all the varieties which have been described as members of the group, *Streptococcus pyogenes*.

Lingelsheim, Kurth, Thalmann, Schottmuller, Smith and Brown, Gordon, Andrews and Horder, and perhaps best known of all, Holman, have offered various classifications based on length of chain, blood laking properties, pathogenicity, fermentation of sugars, and hemolysis together with fermentation of lactose, mannite, and salicin (Holman).

Few, and of these only recently, have authors troubled to classify the anaerobic streptococci. The reason for this lack of classification is not only the fact that knowledge concerning these important agents of disease has been long in coming, but also the fact that study in this direction has been slighted due to lack of attention to and

knowledge of anaerobic methods of study. More recently there has been a rising interest in the anaerobic streptococci especially among the French workers but also to marked degree in the past very few years in this country.

Prevot, (4), in his thesis on the anaerobic streptococci gives a rather complete classification of these organisms. He suggested a preliminary differentiation into three main classes: (a) those which produce fetid and other gases in culture (b) those which do not produce such gas (mostly very small cocci) (c) those which are only "anaerobes of predilection" -- that is they can be educated, so to speak, to aerobic growth. Prevot found this differentiation to be justified by serological tests.

Many authors have attempted to dispose of the problem of classification by making an attempt at an individualistic classification of organisms into strains according to the variations noted within the group of anaerobic streptococci just in their own series of cases.

Colebrook and Hare, (40), offer a practical classification of anaerobic streptococci occurring in puerperal sepsis based on cultural characteristics:

Type A - most common - opaque colonies, 1.5-2.5 mm. in diameter - no hemolysis - cocci the size of aerobic species - long chains rarely seen - unpleasant, fetid odor.

Type B - transparent colonies smaller than A - no hemolysis - usually a "micro" type, 0.3-0.4 microns in diameter - hard to keep alive - do not usually produce gas.

Type C - slower growth - after a week produce coal black colonies on blood agar - very fetid odor.

Type D - very few strains which show hemolysis on blood agar surface - possible a variant of either Type A or B.

These authors state that differentiation is not always sharp enough on the basis of the above classification to identify strains definitely with one type or the other.

Schwarz and Brown, (41), have studied the cultural characteristics of the colonies in their series of cases and have found that their colonies fit into the classification of Colebrook and Hare. They particularly note the actions of the organisms on meat media as to gas production, odor, pigmentation,

and digestion. Digestion is of the utmost importance, they believe, because it is an indication of the proteolytic power of organisms found in individual cases. This is of prognostic significance as usually the greater the proteolytic power, the greater the virulence of the infection.

There is in the literature considerable lack of complete agreement upon the proper method of classification of these organisms. Here and there inconsistencies in nomenclature and description of the anaerobic streptococci have crept into the literature due to earlier lack of complete understanding of the anaerobic nature or the streptococcic morphology of the organisms under consideration and to lack of thoroughness or knowledge in regard to anaerobic technic. Also, even with the extant more nearly satisfactory understanding of the subject, there remain strains which are classed as "doubtful anaerobic streptococci", their doubtful character being in main in regard to whether or not they are justly to be classed as obligate anaerobes, or only microaerophilic streptococci, or facultative anaerobic streptococci.

There is not at present any truly satisfactory

basis of classification of these cocci. The very small size of some of them which led Lewkowicz, (11), to separate one group under the name *S. anaerobius micros* (see section on History), is found to be inconstant - they tend to increase in size upon cultivation in artificial media according to Colebrook and Hare, (40). Gas formation, which is an important feature in Prevot's classification, (34), can hardly suffice for ultimate differentiation until we have found a good medium for their cultivation which is approximately constant in composition. The tendency to divide in more than one plane, which characteristic Prevot utilized also in his method of classification, is probably too indefinite a characteristic to be used for differentiation.

From a bacteriological standpoint the most complete and satisfactory classification of the anaerobic streptococci which I have been able to find to date is that of Ford, (39).

Ford groups together all the anaerobic staphylococci, diplococci, and streptococci, and classifies them as genus 10 under Tribe B (Streptococceae - Trevisan) under Family IV (Coccaceae - Lopf - emended Migula) under Order E or Eubacteriales, under Class

Schizomycetes. Thus they are classified by Ford in a genus separate from the genus, Streptococcus, which, therefore does not include those cocci occurring in chains which live under anaerobic conditions.

Following is a general plan of Ford's Genus 10 including anaerobic cocci other than streptococci and following this is a detailed classification including morphology, staining reactions, cultural characteristics, pathogenic action, etc. of the species pertinent to this study (designated in the general plan by an asterisk):

\*Micrococcus fetidus - Veillon.

\*Kronig's anaerobic streptococcus.

\*Staphylococcus parvulus - Veillon and Zuber.

Diplococcus reniformis - Cottet.

\*Sternberg's anaerobic streptococcus.

\*Streptococcus anaerobius micros - Lewkowicz.

Micrococcus gazogenes alkalescens anaerobius -  
Lewkowicz.

\*Silberschmidt's anaerobic streptococcus.

Diplococcus magnus anaerobius - Tissier and  
Martelly.

Micrococcus A - Grigoroff.

Anaerobic coccus of Gioelli.



\*Streptococcus Schwarzenbeck - Graf and Wittneben.

\*Streptococcus K - Graf and Wittneben.

Diplococcus orbiculus - Tissier.

Jungano's anaerobic staphylococcus.

\*Streptococcus putridus - Schottmuller.

Staphylococcus aerogenes - Schottmuller.

\*Costa's anaerobic streptococcus.

\*Staphylococcus asaccharolyticus - Distaso.

\*Anaerobic streptococcus - Marwedel and Wehrsig.

Bloomfield's anaerobic coccus.

Adamson's anaerobic diplococcus.

Micrococcus minutissimus - Oliver and Wherry.

Hall's micrococcus.

Thomson's anaerobic diplococcus.

Micrococcus fetidus - Veillon

This organism first isolated by Veillon (see historical) from fetid pus in inflamed Bartholin's glands. Apparently same as the organism found by Rist in suppuration of the ear, by Guillemot and Cottet in pulmonary gangrene, by Jeannin in putrid puerperal infections and by Halle in the vagina and in inflamed Bartholin's glands. The coccus found by Norris in liver abscesses was thought by him to

be the same. Menge and Kronig's anaerobic streptococcus may also be the same. According to Veillon it was found in angina and perinephritic phlegmon with fetid pus by Ludwig.

Morphology - small cocci - single, diplococci, small masses, and in culture short chains of three to four diplococci. Sometimes elongated and almost bacillary with pointed ends according to Veillon.

Stains readily with anilin dyes - Gram-positive.

Grows only under anaerobic conditions, feebly at twenty-two degrees C. better at thirty-seven degrees. Deep agar colonies small and round, whitish gray. Uniform turbidity in broth. Slow, scanty growth on gelatin.

Gelatin not liquefied. All cultures yield very fetid gases, smelling like putrid blood or dental caries.

Pathogenic to rabbits and guinea pigs on subcutaneous inoculation - produces cold abscesses in which the organism is present.

Kronig's anaerobic streptococcus

Isolated by Kronig from the vaginal secretions of pregnant women.

Morphology - cocci in chains of ten to twelve organisms exactly like cocci found in puerperal fever.

Stains - Gram-positive.

Grows - easily in 5% grape sugar agar - colonies visible in twenty-four hours about 2 cm. below the surface. Grows also in the above medium to which has been added lactose, sodium formate or indigocarmin - indigocarmin reduced to colorless indigo. Scant growth in slightly acid agar - no growth in fluid media, plain bouillon, or bouillon containing grape sugar or milk sugar. No coagulation of milk. Optimum temperature range from twenty-eight degrees C. to forty degrees C. and no growth below twenty-five degrees C. No growth under aerobic conditions.

Pathogenicity - none to rabbits.

Staphylococcus parvulus - Veillon and Zuber

Obtained originally by Veillon and Zuber from fetid pus in appendicitis. Same species obtained by Guillemot in pulmonary gangrene, by Cottet and Jungano in urinary tract infections, by Rist in

chronic middle ear disease, by Lainer in septic diphtheria, and by Heyde in acute osteomyelitis. Ozaki obtained cultures from material about the teeth.

Morphology - very small cocci, 0.3-0.4 micron in diameter - occurs singly, in pairs, in bunches, and in short chains.

Stains feebly with methylene blue, best with carbol-fuchsin. Gram-negative. Claudius negative.

Grows - only as an obligate anaerobe - optimum temperature thirty-seven degrees C. - slow growth at twenty-two to twenty-three degrees C. - grows well on most media but best on neutral or weakly alkaline media - grape sugar favors growth - organisms viable at first for fifteen to twenty days in sugar agar at thirty-seven degrees C., later only four to five days. (Ozaki)

-Agar plates - surface colonies small, very fine points, very transparent and slowly disappearing. Deep colonies well defined and limited, yellowish, granular at first. Small amount of gas evolved

which cracks the agar feebly and is very fetid. (Veillon)

For detail in regard to characteristics on other types of media see Ozaki, (42), or Ford, (39-page 451).

Pathogenic action - subcutaneous injection of large doses produces abscesses in mice and guinea pigs. These are lentil to pea sized and contain thick yellow pus which does not stink. In rabbits subcutaneous injection produces infiltration but not abscess formation.

Sternberg's anaerobic streptococcus

Isolated by Sternberg from the sputum in a case of actinomycosis of the lung.

Morphology - fairly long chains made up of oval or round, fairly plump cocci of various sizes, in general like a streptococcus but two to three times as large.

Stains - Gram-positive.

Grows - only under anaerobic conditions.

Agar slant - delicate fine expansion made up of small, discrete colonies.

Agar stab - scanty development.

Plain agar plates - scanty development in

the form of small colonies, microscopically appearing granular with wavy borders and radial striations in the periphery.

Sugar agar plates - small colonies about the size of a pin-head, microscopically dark brown to black, with irregular surface and smooth edges from which many prolongations extend peripherally.

Bouillon remains clear with a scanty sediment.

Sugar Bouillon - abundant sediment with small granules along the wall of the test tube.

Gelatin stab - fine, veil-like turbidity in the depths at room temperature, with small granules about the size of pin-heads.

Loeffler's blood serum - small, slightly raised, moderately moist colonies, pin-head in size.

Potato - slightly acid (not neutralized), shows no growth or very small white granules. Microscopically potato covered with organisms which could be transferred for

at least a month.

Pathogenicity - subcutaneous injections in rabbits produces localized nodules made up of granulation tissue.

Streptococcus anaerobius micros - Lewkowicz

Described by Lewkowicz who found it in the mouths of nurslings.

Morphology - very small cocci (0.25-0.4 micron in diameter), often elongated or lanceolate. Many involution forms. Bacillary forms in sugar agar. Usually arranged as diplococci or short chains. Gram-positive.

Grows only at thirty-seven degrees C. as a strict anaerobe - lives two to three weeks in cultures - killed at sixty degrees C. for fifteen minutes.

In deep sugar agar growth appears in two to three days, ten to fifteen mm. below the surface - made up of small colonies 0.05 to 0.07 mm. in diameter - microscopically round, finely granular and transparent or with short filamentous prolongations. Isolated colonies are two to three mm. in diameter under the micros-

cope, whitish, round or oval, opaque, often with similar filamentous prolongations - rarely colonies are 0.6 to 0.8 mm. in size - surface colonies when isolated are 0.25 to 0.3 mm. in diameter after forty-eight hours, later 0.8 mm. in size. They are round, transparent, grayish, slightly elevated, microscopically finely granular.

Broth - turbidity with an abundant powdery deposit.

Milk - no coagulation.

No gas from cultures but a well marked odor.

Pathogenicity - practically non-pathogenic.

Silberschmidt's anaerobic streptococcus

Isolated by Silberschmidt from a fatal case of gas gangrene in association with staphylococci, streptococci, and bacilli.

In the pus it appeared as a typical streptococcus in short and long chains. In cultures the organisms were small and usually stained with difficulty. In certain cultures as in anaerobic surface agar, the cocci stained typically, in short curved chains here and there enclosed in a capsule.



Morphology - see above.

Stains - usually gram-positive.

Grows - only as an obligate anaerobe, the growth resembling that of a streptococcus or diplococcus. Growth more abundant in broth than in sugar broth, after two days giving a sediment and a fine deposit along the walls. There is a marked unpleasant odor to the cultures.

Agar slant - small, sharply limited, transparent, colonies, punctiform to pin-head in size - abundant growth in water of condensation.

Agar stab - growth in the depth with wavy edges - no isolated colonies.

Milk - no coagulation.

Gelatin - no growth.

Pathogenicity - non-pathogenic to mice and guinea pigs.

Streptococcus Schwarzenbeck - Graf and Wittneben

Described by Graf and Wittneben from a skin abscess suspected of being actinomycosis. Gram stained films from the purulent material revealed regular cocci in pairs or in short chains together

with Gram-positive rods.

Morphology - cocci or short oval rods like those seen in ordinary streptococcus cultures, the cocci being usually in chains. In neutral bouillon the chains were composed of thirty to fifty elements, in alkaline bouillon of only about eight elements. The chains were frequently wound and tangled. The individual elements were smaller than ordinary Streptococcus pyogenes.

Stains - best by Gram, less well with methylene blue, somewhat better with carbol-fuchsin (1-10). No Neisser granules - not acid-fast - no capsules - no flagella.

Grows - best under anaerobic conditions - some aerobic development on media containing serum and sugar. Optimum temperature twenty-five degrees C. - no growth at twenty-two degrees C. Vitality on the surface of media about two to three weeks, in condensation water of Loeffler's serum about eight weeks. Very slight growth on litmus-lactose agar of v. Drigalski and Conradi, due possibly to the nutrose present. On agar which contains only serum or only sugar

no superficial growth under aerobic conditions. Deep agar shake cultures show above a colony-free zone, fifteen mm. deep, sharply defined, then a zone seven mm. deep where the agar is thickly studded with colonies, then again a zone about six mm. deep with no colonies, and finally a zone of abundant development at the bottom of the tube. This streptococcus seems to develop in two different concentrations of oxygen. All surface colonies stick closely to the media, leaving a defect in the medium when removed.

Cultural characters - neutral agar plates show no superficial colonies under aerobic conditions. Deep colonies in seventy-two hours under a magnification of sixty diameters are black brown, flaky with yellow, with rosette-like toothed edges. After five days a yellow honeycomb-like zone appears about the colonies. In an atmosphere of hydrogen yellowish, round, finely granular, superficial colonies with sharp edges developed.

Agar stab - definite development in the depths in twenty-four hours; delicate, thread-like,

not characteristic. In forty-eight hours very faint superficial expansion.

Deep agar shake - upper zone, nine to ten mm. deep, free of colonies; below, round, irregular whitish colonies in forty-eight hours.

Acid agar plates - no growth under aerobic conditions. In an atmosphere of hydrogen the colonies appear under a magnification of sixty diameters as coarsely granular, round expansions, with irregular, coarsely granular edges. Deep acid agar shake - moderate development in the depths only with rich inoculations. Above, a colony-free zone fifteen mm. deep.

Grape sugar agar - growth like that in neutral agar, but somewhat more abundant.

Glycerin agar - growth like that in grape sugar agar.

Serum agar (one to five) - no superficial colonies under aerobic conditions. Deep colonies appear in forty-eight hours which are whitish with irregular edges. With a low magnification (sixty diameters) they are blackish-brown, rosette-like, flakey. Growth in atmosphere of hydrogen like that in neutral

agar.

Shake cultures show the typical four zones.

Grape sugar serum agar - under aerobic conditions superficial colonies, round, succulent, yellowish-white, size of pin-heads. Under low magnification (sixty) blackish-yellow, granular, with fairly sharp edges. With a magnification of five hundred diameters cocci in chains appear long and oval.

Grape sugar serum agar shake - whitish colonies arranged in zones, most abundant in the depths.

Milk agar (one to one) - superficial colonies in forty-eight hours sparse, snow-white, with faint zones. Under low power black, round, edges flakey and irregular. Better development in the depths.

Milk agar shake - colony-free zone five mm. above; below, snow-white, irregular, roundish colonies, larger than those of *Streptococcus pyogenes*.

Blood agar (one to ten) - superficial colonies barely visible in forty-eight hours, very delicate, punctiform, whitish. No hemolysis.

Loeffler's serum - punctiform pure white

colonies in forty-eight hours. In seventy-two hours up to one mm. wide, not definitely round, edges clearly irregular, central portions raised, button-like.

Drigalski-Conradi agar - trace of growth in three days with rich seeding. Colonies reddish-white or light red with dark red centers by transmitted light. Same type of growth when medium contains no crystal violet.

Drigalski-Conradi agar with serum added - superficial colonies in forty-eight hours reddish-white, somewhat succulent, not definitely round, edges indented, narrower by transmitted light, with lighter reddish-white edges, middle portions bluish-red. With a magnification of sixty diameters the colonies are round, rosette-like, with light edges. A magnification of five hundred shows thick oval rods in chains and Y formation as well as chains of cocci, containing up to twenty elements. Better growths in the depths. Medium red throughout. Potato - no visible growth.

Gelatin - no growth at twenty-two degrees C.

At twenty-five degrees C. no superficial growth.

In the depths, colonies appear under low magnification black, flakey, rosette-like. Periphery yellowish. No liquefaction.

Gelatin stab - thread-like growth, later showing white button-like masses. Growth in gelatin at thirty-seven degrees C. under anaerobic conditions. Gelatin liquefied by a ferment. More abundant growth in serum gelatin, only at thirty-seven degrees C.

Bouillon freshly boiled shows slight development in twenty-four hours under both aerobic and anaerobic conditions. More abundant in forty-eight hours. Granular masses along the glass, sinking to the bottom, bouillon clear, yellowish-white granular sediment.

In bouillon not covered with paraffin, growth along the glass begins one cm. below the surface.

Grape-sugar Bouillon - somewhat more abundant growth.

Acid Bouillon - no growth.

Alkaline Broth - more abundant growth than in neutral broth.

Neutral Broth - under a magnification of five

hundred shows chains of twenty to thirty elements beautifully wound. Alkaline broth shows chains with only eight elements and ovoid rods in V forms.

Peptone Water - faint growth. No indol.

Potato Bouillon (Wrzosek) - growth like that of *Streptococcus pyogenes*.

Milk - clotted in four days to fairly firm masses. Under a magnification of five hundred diameters short chains and diplococci appear. No clotting at twenty-two degrees C.

Litmus Milk-whey - reddening begins in seven days, outspoken in four weeks, not increasing, slight sediment.

Barsiekow's Milk Sugar Medium - reddening, gradually becoming deeper, marked in six weeks, opalescent by reflected light.

Barsiekow's Grape-sugar Medium - marked reddening in forty-eight hours, strong in fourteen days, somewhat opalescent in reflected light. No clotting. Clotting in one and a half to three months.

Pathogenic Action - non-virulent to mice, guinea-pigs, and rabbits by intraperitoneal



inoculation. Subcutaneous inoculation produced in guinea-pigs nodules up to cherry size, disappearing after six to eight days. On incision the nodules were found to contain pus. Similar nodules produced in mice, lentil- or pea-sized. Abscesses also produced in rabbits.

Streptococcus K of Graf and Wittneben

An anaerobic streptococcus cultivated by Graf and Wittneben from the pus of a brain abscess. In its general characters it resembles *Streptococcus Schwarzenbeck*.

Morphology - very small cocci in chains and in small masses.

Stains positively by Gram's method.

Grows - only sparsely under aerobic conditions - good under anaerobic conditions - good in an atmosphere of hydrogen.

Cultural Characteristics - no hemolysis on blood agar plates - in deep agar shake cultures of neutral grape sugar and glycerin agar, a colony-free zone five to seventeen mm. appears above, and below this a zone of thickly distributed colonies. Better growth on media containing grape sugar and on acid media -

considerable acid produced in cultures, milk acidified and clotted in forty-eight hours - reddening of litmus milk. Colonies cling to the surface of solid media, leaving a defect in the medium when removed.

Streptococcus Putridus Schottmuller

Obtained by Schottmuller from a great variety of cases including otitis media, meningitis, empyema, endometritis, salpingitis, peritonitis, etc. Apparently the same organism was isolated earlier by Menge and Kronig from pathological conditions in the puerperium in which stinking pus was present.

Morphology - diplococcus forms and longer and shorter chains of streptococci from the infected tissues. In artificial media short and long twisted chains made up of oval elements arranged in pairs as diplococci. In old cultures the organisms appear as rods and granules of very different sizes.

Stains - with usual anilin dyes. Gram-positive.

Grows - only as an obligate anaerobe under very complete anaerobic conditions. These conditions are best obtained in deep agar,

either in stabs or shake cultures. The addition of reducing substances favors the growth of the organisms. Cultivated well also in broth containing blood where apparently the organisms at the bottom are protected from oxygen.

Ordinary anaerobic methods, such as the use of pyrogallic acid, do not suffice for cultivation. No satisfactory growth in ordinary broth.

Cultivated on blood agar plates in an atmosphere of hydrogen. Growth best obtained in shake cultures in sugar agar containing blood, the blood being added to the melted agar at forty-five degrees C. The colonies develop in about twenty-four hours and the agar is full of gas bubbles. Grows best at thirty-seven degrees C. No growth at twenty degrees C.

Vitality poor. Transfers successful for only fourteen days.

Agar plates - on plain agar plates in an atmosphere of hydrogen, the colonies are small and gray, like those of the ordinary streptococcus.

Agar stabs - in twenty-four to forty-eight hours the colonies develop in the anaerobic zone, small, grayish-yellow, whetstone in shape. Similar colonies in shake agar cultures.

Indigo and Neutral Red Agar - good growth, the latter showing decolorization and fluorescence.

Ordinary Agar or Sugar Agar - gas formation does not occur, but does occur in blood agar. The gas is H<sub>2</sub>S, stinking and inflammable.

Blood Agar - colonies small, gray-white, resembling muscle trichinae. Later they are pin-head in size. The cultures give off a peculiar, disagreeable odor. Colonies on blood agar plates porcelain white, the size of pin-heads. Deep colonies are glistening white. No hemolysis. No gas in sugar agar.

Milk - good growth. No clotting.

Blood Bouillon Cultures - the putrid odor is especially perceptible when the blood pigment takes on a peculiar light red shade. The spectroscope shows H<sub>2</sub>S. After about ten days the bouillon cultures are black. Blood from infected cases and pus also has the disagreeable odor.

Non-pathogenic to rabbits and guinea-pigs.

Remarks - this organism is regarded by Schottmuller as the causative agent in fetid

infections in which stinking gases develop such as the thrombophlebitic form of puerperal sepsis (Putrescentia uteri), secondary local purulent processes such as pyosalpinx, abscesses in Douglas' pouch, etc. It is frequently the cause of lung gangrene, causing a destruction of lung tissue and a disagreeable odor.

Costa's Anaerobic Streptococcus

Found by Costa in a large abscess in the abdominal region and associated with Vincent's fusiform bacillus.

Morphology - in pus a very small coccus in long chains like a streptococcus.

Stains - does not stain by Gram's method.

Grows - cocci in chains develop on media under anaerobic conditions.

Cultural characteristics - in broth it produced a slight turbidity with a whitish sediment. Gelatin was slowly liquefied, milk not coagulated.

Staphylococcus Asaccharolyticus-Distaso

Isolated from the intestinal contents by Distaso.

Morphology - a diplococcus, a short-chained

streptococcus with four to eight individuals, or very large bunches. Individual cells are twice as large as those of the staphylococcus of Jungano.

Stains - Gram-positive.

Grows - only as an anaerobe.

Sugar Agar - colonies about as large as grains of sand; when separate, growing to the size of a pin-head. Microscopically they are transparent like a drop of liquid vaselin. Cultures emit a bad odor.

Milk - acidified. No coagulation.

Gelatin - grows at thirty-seven degrees C. without peptonizing it and forming a precipitate like cotton wool at the bottom of the tube.  
White of egg - grows without attacking it and producing viscid zoogloea.

Sugars - no action.

Indol - positive.

Regarded by the author as like the staphylococcus isolated by Jungano.

Anaerobic Streptococcus of Marwedel and Wehrsig

Found in two cases of gas gangrene by Marwedel and Wehrsig.

Morphology - Streptococci, often in lacy chains (six to ten diplococci).

Stains - Gram-positive.

Grows - only as a strict anaerobe.

Blood broth - according to Weinburg and Seguin it turns black and produces a putrid odor.

Glucose agar made from human placenta - gives gas, the gas, however, coming from the proteins in the medium.

Cultures - the organisms died out rapidly.

Pathogenicity - virulent to guinea-pigs, one c.c. killing them in twelve hours by subcutaneous inoculation. At autopsy an extensive gelatinous hemorrhagic edema infiltrated with gas was found. Lesions not putrid. Pathogenic power lost rapidly.

Probably the same as Streptococcus putridus of Schottmuller.

### C. Habitat and Pathogenic Activity

Although laboratory animals are not often killed by the inoculations of pure cultures of anaerobic streptococci, definite pathogenic effects have been observed and recorded by several workers.

Harris and Brown, (36), state that three of their fifty-seven strains obtained in a study of puerperal fever killed mice within twenty-four hours. Marwedel and Wehrsig, (43), with a strain isolated from a war wound, produced a fulminating infection in a guinea pig which killed the animal in twelve hours. Prevot, (34), obtained pathogenic effects: "phlegm on gazeux", gelatinous edema, abscess formation, and sometimes death with all of his types of the organism. He found that cultivation in media containing fresh tissue or blood enhanced the pathogenic activity, while that property was usually lost by cultivation on the ordinary media. Wegelius, (44), reported the production of small abscesses in the peritoneums of mice. Colebrook and Hare, (40), report the production of small caseous lesions at the site of subcutaneous injection. They state that none of the animals died. These men also report the results of inoculation of primary cultures from the blood of



puerperal fever patients into animals. In two of the animals definite infections were caused and one case of fatal septicemia was produced in a mouse. In a guinea pig a large abscess formed at the site of inoculation, and in another mouse a large splenic abscess occurred which appeared to have originated from an infarct. Anaerobic streptococci were recovered from this abscess.

In twenty-three cases reported by McDonald et al, (45), in which anaerobic streptococci were recovered at the autopsy table, some part of the intestinal tract was involved nine times, the lung primarily seven times, and the meninges four times. In these cases the lesions occurred in various tissues. Abscess of the lung was the most prominent pulmonary lesion in four cases. Gangrenous appendicitis with rupture accounted for five of the intestinal lesions. These were usually followed by the formation of an abscess. There were three cases in which carcinoma of the colon had perforated and produced an abscess. One case was a perforated duodenal ulcer which had been surgically closed and was followed by a huge subdiaphragmatic abscess with empyema on the right side and multiple chronic abscesses of

the liver. The duodenal lesion had healed and its site was marked merely by a scar. The four cases in which anaerobic streptococci were isolated from the meninges represents a type of infection about which practically nothing has been written. The focus in each of the cases was different. The foci represented chronic infection of the nasopharynx, frontal sinus, and middle ear. In one case an infected wound in the region of the tenth thoracic vertebra had served as a focus.

In general the most of the lesions were granulomatous in character microscopically. They presented the appearance of a non-specific granuloma. This was not true of the cases which terminated rapidly. In these cases extensive necrosis was the most significant finding.

Anaerobic streptococci were recovered in pure culture in forty-eight per-cent of the cases studied. Often they were isolated both from the blood and from the lesions. These authors believe that the frequency with which they are found in pure culture emphasizes the importance of the anaerobic streptococci as a cause of human inflammatory disease and suggests that they played an important role in all the cases in

which they were present whether they were the sole pathogens present or not.

The duration of the major symptoms varies from four days to over a year. In the minority of cases does the terminal illness last less than one week. This chronicity is apparently attributable to the low virulence of the anaerobic streptococci.

The pathogenicity of the anaerobic streptococci for man is difficult to evaluate with any degree of certainty.

In spite, however, of the comparatively small amount of attention paid these organisms, they have been found by many workers to occur in a rather wide variety of pathological conditions and also in many situations of apparently normal nature in which the organisms are apparently non-pathogenic and of a saprophytic character.

Anaerobic streptococci have been found in chronic ulceration of the lip, in perinephritic abscess, urinary abscesses, periurethral gangrene, pyonephrosis, and cystitis, in the vagina, vulva, uterus, tubes, ovaries, parametrial tissues, and pelvic veins in puerperal infection, in cases of vulvo-vaginitis, Bartholinitis, suppurative salpingitis, and periuterine abscess, in peritonitis, in appendicitis, in cholecystitis, in liver abscesses, in operative wounds, in

septicemia and bacteremia, in acute osteomyelitis (traveling here via blood from the lungs), in Ludwig's Angina, in purulent infections of the ear (both external and middle), in putrefactive and gangrenous purulent processes of the respiratory tract including the so-called putrid pleurisies, pulmonary gangrene, and empyema, in ulcers of the skin and subcutaneous tissue, and skin abscesses, in gas gangrene, in brain abscess, and in meningitis. They may play a part in auto-intoxication due to their action in the putrefaction of the contents of the intestine.

Anaerobic streptococci have been found in non-pathogenic situations as saprophytes occurring on the mucosa of all the natural cavities of the human body. In the normal or non-infected body they have been found in the vagina, uterus, intestines, extra-hepatic bile ducts and gall bladder, in the mouths of nurslings, and from the area around the teeth in adults. They have been isolated from the feces.

One simple explanation of the role of the anaerobic streptococci in gangrenous and necrotic processes is that of Prevot, (4), the essence of which is briefly set forth in the following few words:

The anaerobes live in the natural cavities; they

move into a neighboring lesion, rapidly multiply and invade the process, thus proclaiming their proper pathogenic role, resulting in gangrene of the lungs, genitalia, appendix, biliary tract, mouth, ear, etc.

#### D. Anaerobic Culture Methods

On going through the literature, one is impressed by the variety of principles and devices which have been used over a period of years in trying to arrive at a really practical and satisfactory method for the isolation and culture of anaerobic organisms.

Various devices for the removal of oxygen from air-tight containers or jars have been employed. Perhaps the most satisfactory and most well known of these anaerobic jars is that of McIntosh and Fildes. An improved model of this is described in an article by these men in 1921, (46). This is an air-tight jar which utilizes palladium-asbestos as an agent for the removal of oxygen continuously after the culture has been placed within the jar. Diagrams of the jar and a description of its principle and details of its use are to be found in this above cited article.

Other types of anaerobic culture such as poured plates, surface inoculation into plates, and liquid media for the culture of anaerobes are described in the literature. In a majority of the

articles on the subject of anaerobic organisms are to be found almost as many different methods of culture, each author having his own pet variations to offer.

The literature on methods of isolating and cultivating the obligately anaerobic bacteria has been very completely reviewed by Hall, (47), with a bibliography of 247 citations.

The consensus of opinions as to the most certain manner of isolation of these organisms is by the use of the single cell pipette, but this method involves much waste of time and materials, and a considerable degree of proficiency in technique. Its use, therefore is practically limited to a specialist in this field.

Much ingenuity has been expended, therefore, in attempting to devise a plating method by which colonies may be isolated either by poured plate or surface inoculation. Hall, (47), has cited at least twenty attempts of this nature.

Such plates are designed as self-contained units and practically all make use of the alkaline pyrogallate method of oxygen elimination. Many of these are too cumbersome or so complicated that

they cannot be, or at least have not been, simplified to a point where they may be feasibly produced commercially and so are not available. Furthermore, in most of these devices the alkali and pyrogalllic acid are mixed and then an attempt is made to quickly seal the culture in. But herein lies a major disadvantage which is that before the seal is accomplished, an unknown but probably quite considerable amount of reaction has taken place. To overcome this, various tricks have been resorted to such as placing the ingredients separately into capsules, cardboard, or cotton and placing them thus into the culture to be mixed after the seal has been accomplished. This method, it can readily be seen, offers new avenues for the contamination of the culture and has not been very widely accepted.

The device described by McLeod, (48), in which a special plate is inverted over a divided porcelain capsule suggests a further modification. This principle has been used in a very satisfactory anaerobic dish described by Spray, (49), but with further simplification so that the dish could be produced on a commercial basis and become generally available.



Spray's dish is composed of two parts; the top being the ordinary 100 mm. Petri dish lid, the bottom being a special annealed deep glass dish with an uprolled edge forming a moat for the paraffin or plasticine seal. The bottom of the dish is divided by an impressed ridge of sufficient height to allow each side of the divided bottom to contain 10 cc. or more of fluid. In use, the cultures are inoculated into melted caseindigest or dextrose agar by the usual dilution method, after driving the oxygen from the tubes by boiling. The dishes are inverted on a chilled surface and the culture poured into the lid of the dish. If the medium is first cooled to forty-five degrees C., the agar should set within one minute without the formation of condensation water which interferes with single colony development. The dish is then reversed and with separate pipettes the two solutions are placed in opposite compartments of the deep dish. The moat is then sealed with hard paraffin or plasticine, after which the dish is tilted to mix the solutions, and incubated at the desired temperature. For further detail on this method, the reader may, if

he so desires, refer to the article by Spray cited above.

More recently Brewer, (50), in 1940 has made use of several well established principles and has developed a clear, liquid medium, simple to make and simple to use which affords a reliable method for the culture of not only anaerobes, but micro-aerophils and aerobes as well. Although other media have been used successfully for the "aerobic" cultivation of anaerobes, Brewer and others claim that this is the first clear, liquid medium which will remain anaerobic over long periods of time without the supplementary use of a seal or other special apparatus. This medium will remain anaerobic for more than one month when used in regular culture tubes without any type of seal. Tubes of this medium which were stored for a month at room temperature and then inoculated with a variety of anaerobic organisms gave growths as good as those of control cultures in anaerobic jars. This long period of anaerobiosis is far in excess of the seven day period of incubation used in testing the sterility of biological preparations. This medium has the unusual property of again assuming an

anaerobic state if it has been aerated by stirring or shaking. It may be prepared in dehydrated form and has general utility.

Glucose or pork infusion broth containing 0.05% agar is used as a base for this medium. Hitchens, (51), Spray, (52), and Falk, (53), have shown that small numbers of organisms grow more readily if small percentages of agar are used, and as Brewer stated "It is realized of course, that even this small percentage of agar lessens convection currents, thereby prolonging the anaerobiosis obtained without noticeably affecting the fluidity of the medium." To insure proper oxidation-reduction potential, 0.1% of sodium thioglycollate is added. An Eh indicator may be used to give color to any portion of the medium when it becomes aerated. For this purpose Methylene Blue 0.0002% is used. The sodium thioglycollate, in addition to maintaining a desirable oxidation-reduction potential, combines with and inactivates most of the mercurials used as preservatives. Because of this property the medium is much more likely to promote the growth of organisms from contaminated biologicals which are preserved with one of these metallic, bacterio-

static compounds. This property is of value also in cultivating organisms which have been exposed to antiseptics.

The tubed Brewer's medium which is available from the Baltimore Biological Laboratory is a liquid medium the surface of which is aerobic while the subsurface is anaerobic; hence the medium possesses a variable degree of anaerobiosis, making possible the cultivation in one medium of anaerobes as well as micro-aerophils and aerobes. For this reason it has been termed a facultative medium.

This medium has been used for some time by Dr. Millard F. Gunderson, (54), at the College of Medicine and University Hospital of the University of Nebraska with very satisfactory results.

In the cultivation of anaerobic organisms, besides strict attention to the suitability of the medium, it must be borne in mind of course that, here as well as with other organisms, knowledge and care as to the proper site in the lesion from which to obtain the culture and as to the prevention of contamination of the culture must be in force.

### E. Criteria For Anaerobiosis

In most of the papers written the authors do not give the criteria by which they judged the organisms to be strict anaerobes and in some of the earlier ones it is at least possible that the capacity of certain strains to grow aerobically after one or two subcultures was not fully recognized. The introduction of the anaerobic jar has greatly facilitated the study of surface cultures. Also the newer media for anaerobic culture have simplified this matter.

Elizabeth White, (59), in two series of fifty cases each used the following criterion for strict anaerobiosis: cultures were made from vaginae on two blood agar plates (five per-cent citrated horse blood in nutrient agar). The plates were incubated for forty-eight hours, one aerobically and the other anaerobically [in a McIntosh-Fildes Jar, (46)]. Streptococci found on the anaerobic plate were subcultured onto blood agar plates for further aerobic and anaerobic incubation, a second aerobic subculture being made after forty-eight hours if the first one showed no growth. If the

organism still failed to grow aerobically, it was considered to be an obligatory anaerobe.

## CLINICAL ASPECTS

In the section on "Habitat and Pathogenic Activities" has been included a comprehensive list of the many pathological conditions of the human body in which the anaerobic streptococci have been found as sole or at least contributing causative factors.

It is my intention here not to attempt an exhaustive treatment of all the conditions included in that list nor, for that matter, of any one of them, each of which could well be in itself a subject suitable for a thesis longer than this one. It is my purpose here to present only the salient features of a few of the more common or more important conditions listed as they may have an important relation to the subject of the anaerobic streptococci.

### A. Puerperal Infection

In 1843 Oliver Wendell Holmes, braving the medical Podsnaps of his day published his epoch-making paper on puerperal fever.

Mr. Podsnap you recall, was that very respectable and highly self-satisfied individual in Dickens' "Our Mutual Friend" who "settled that whatever he put behind him he put out of existence". There was a dignified conclusiveness - not to add a grand convenience - in this way of getting rid of disagreeables which had been so important in establishing Mr. Podsnap in so high a place in Mr. Podsnap's satisfaction.

"I don't want to know about it; I don't choose to discuss it; I don't admit it."

Braving then the Podsnappery of his generation, Holmes said, "The disease known as puerperal fever is so far contagious as to be frequently carried from patient to patient by physicians and nurses."

Thirty-five years later Pasteur first demonstrated in the blood of a puerperal fever case the organism later to be known as the streptococcus and as one of the most virulent of the bacterial causes



of disease.

In 1867, nineteen years later, Lord Lister first set forth the principles of antiseptis whereby the incidence of puerperal fever as well as other infections was greatly to be curbed and prevented.

There has for a long time been discussion as to whether or not puerperal fever is a disease due to a specific organism like typhoid, diphtheria, etc., or a condition caused by the introduction, fortuitously, perhaps, into the uterus and from there into the circulating blood of any of the organisms normally present in the vagina.

Without doubt in many cases the saprophytic organisms of the vagina have etiological roles thrust upon them by accident. There are still other cases in which, despite the clinical evidence of bacteremia, blood cultures are sterile, due, as has been suggested, to the fact that anaerobic methods are not routinely used, (55).

Although anaerobic streptococci were described by Kronig as early as 1895, very little has been written about those organisms and the part they play in puerperal fever.

In a study of the bacterial flora of the

vagina, Kronig, (23), in 1895 demonstrated the presence of anaerobic streptococci, a finding fully corroborated by the more extensive investigations of Natvig, (26), and Wegelius, (44), in 1908. At first regarded as saprophytes, though later known as parasites, the importance of these organisms in the production of puerperal fever was first emphasized by Schottmuller in 1910, (27). (See History)

In 1926, Schwarz and Dieckmann, (56), commented upon the paucity of American medical literature on this subject, finding up to that time only the report of Little, (57), and but few references in the English language. The interest of these men in the anaerobic organisms was stimulated by the frequency of negative cultures (by aerobic methods) in cases which they felt sure were infected. These men published an article in 1926 (supra) in which they described the work of Schottmuller in some detail.

Schwarz and Dieckmann, (29), again published an article on this subject in 1927 in which they reviewed the work on this subject and expressed the feeling that anaerobic streptococci play a considerable role in puerperal infection.

Harris and Brown, (58), reported a study of "fifty uteri cultured at Caesarian section". In fourteen of these uteri streptococci were found and eighteen strains were isolated and studied. Of these eighteen were strict anaerobes and would not have been detected by ordinary aerobic methods of culture. Two strains were micro-aerophilic and the remaining four were facultative strains. They presented in detail their bacteriological studies of these organisms and concluded that certain outstanding features may be recognized when the eighteen strains are regarded broadly:

1. The predominance of anaerobic streptococci.
2. The predominance of the gamma type of organisms in blood agar; the presence of beta types and the absence of the alpha type.
3. The absence of *S. fecalis* and other mannite fermenters indicating that perhaps the streptococci found in the uterus are not of fecal origin.
4. The low pathogenicity of these streptococci for mice and rabbits.

Soule and Brown, (38), in 1932 report the

following from a study of the "Anaerobic Streptococci in the Vagina of Normal Clinic Patients":

1. Aerobic hemolytic streptococci were not isolated in any of 207 cultures from the vaginae of normal pregnant women.
2. Anaerobic growth was noted in sixty percent of all patients.
3. Anaerobic streptococci were isolated in 40% of all cases.
4. Anaerobic streptococci were noted definitely more frequently in primiparae than in multiparae.

Elizabeth White, (59), in 1933 in a study of two series of fifty cases each, reports finding anaerobic streptococci in at least 30% of vaginae at the end of pregnancy and during the first stage of labor. She states that regarding the etiology of puerperal and post-abortum infections with anaerobic streptococci it is significant that 30% or more of women harbour these organisms in the vagina at the end of pregnancy and it is becoming clear that these infections are more likely to occur in patients who have been delivered by instrumentation or have been subjected to internal interference during labor. If anaerobic streptococci are present

in the vagina, they could hardly escape being im-  
planted onto the lacerated tissues of the genital  
tract.

Brown, (60), in an article in 1935, presented  
a table showing the incidence of anaerobic strepto-  
cocci in puerperal infection.

Type of Infection	No. of deliveries		No. of cases of P. inf.			No. of deaths	
	No. of Cases	Aerobic Bacteria	Anaer- obic Bacteria	Mixed	Neg. Culture	Mortal ity	
	12,260	10,879		187			28
Acute Endometritis	175	33	103	38	1	0	
Pelvic Cellulitis	30	8	27	7	1	0	
Peritonitis	21	4	8	9	0	14	
Pelvic Abscess	15	5	5	4	1	1	
Pelvic Thrombophlebitis	20	3	13	1	3	4	
Septicemia	40	15	15	10	7	9	
Suspected Endometritis	23	0	0	0	23	0	
Anaerobic organisms in 80.5% of Endometritis							

Regarding the importance of a bacteriological study of and the proper classification of the type of puerperal infection present, Lash, (61), concludes as follows: that although a clinicopathologic diagnosis gives an anatomic description of the stage of the puerperal infection it is not complete when lacking bacteriologic information which will bring about more accurate mortality statistics, prognosis, and more rational therapy.

Little, (57), as far back as 1905, stated that it was the custom at Johns Hopkins to examine uterine contents bacteriologically in every case where the temperature reached 102 degrees F. during the puerperium and to take cultures after the third stage of labor if a patient entered the hospital with a temperature or had been subjected to attempts at delivery by persons not associated with the hospital. Also in cases of incomplete abortion, especially if of criminal character, cultures were taken before any vaginal examination was done. Anaerobic studies were included in this routine.

The above observations are in line with the belief of Schwarz and Brown, (41), who state that the main problem in dealing with puerperal infection

has to do with keeping down anaerobic infection because infections due to other pathogens may be well controlled merely by observing proper obstetric technique. At the end of a ten year study these workers concluded that:

1. Anaerobic streptococci were by far the most frequent offenders in their series of cases.
2. The above is true for endometritis and other lesions (pelvic cellulitis, peritonitis, pelvic abscess, and septicemia).
3. Anaerobic streptococci were found in 40% of vaginas at term so it is obvious that these organisms are not introduced but rather give rise to endogenous infections and the circumstances predisposing to infections by these organisms are: prolonged labor in which the tissues have been bruised considerably; cases in which the membranes have been ruptured for some time before delivery; and difficult operative deliveries.

### Symptoms, Signs and Clinical Course

Colebrook, (30), reports in his series of cases that in no case was the infection a fulminating one. In the most rapidly fatal case the patient died in eleven days with broncho-pneumonia. The average course whether ending in death or recovery was six to seven weeks and one patient was still in the hospital after twenty-seven weeks illness.

The clinical features as reported by Colebrook were various in nature but in general he summarized them as follows: the patients are much less ill and the fever less severe and less sustained than in the case of the generalized infections with hemolytic streptococci; rigors are frequent, but the general condition is good and the pulse rate often low in the intervals; there is usually little pain, and very little can be learned from physical examination, except perhaps a slight tenderness on deep palpation over the pelvic brim -- a sign which can often be related at necropsy to a septic thrombus in the ovarian veins. In the prolonged cases anemia is a striking feature, and it is progressive. Diarrhea is uncommon except when suppuration has occurred in



the retroperitoneal tissues of the floor of the pelvis. In several cases pus was found to have formed in the above situation. Peritonitis may occur as a direct result of the bursting of an abscess.

The mortality rate in this series was in the neighborhood of 40%.

Necropsy findings in the fatal cases reported were as follows:

1. Septic thrombus in left ovarian vein, extending to renal and inferior vena cava. Abscesses in cave of Retzius and behind left ovary. Foci of suppuration in wall of uterus. Infarcts in kidney and lung.
2. Septic thrombus in right ovarian vein which had ruptured and was communicating with a large abscess behind the caecum. Splenic infarcts, pus in right pleura, terminal endocarditis.
3. Septic thrombus in left ovarian and renal veins. Abscess around left ovary and in uterine wall. Small ulcers in colon and rectum.

4. Retroperitoneal tissues of pelvis honey-combed with foci of suppuration. Pus in both ovaries. Lung infarcts. No septic thrombus found.
5. Septic thrombus in right ovarian vein and inferior vena cava. Thrombus (septic ?) in common femoral vein. Abscess of lung. No other infarctions.
6. Sloughing ulcer of endometrium. Abscesses in pouch of Douglas and uterine wall. Infarcts in spleen and lung. Pleural effusion.

In the past it has been contended that the hemolytic streptococci were responsible for nearly all the severe and fatal cases of puerperal sepsis. The work of Schottmuller, Sommer, Curtis, Hall, Brown, White, Lash and De Costa, Harris, Kronig and Menge, Wegelius, Natvig, Schwarz and Dieckmann, Colebrook and Hare, and others show that that contention must be modified, and that a somewhat different conclusion would have been arrived at if proper methods of anaerobic study had been brought into play earlier.

Colebrook states that two-thirds of his

cases of septicemic infection in puerperal fever cases have been shown to be due to anaerobic streptococci and only one-third to *S. pyogenes*.

#### Treatment

In regard to treatment of the puerperal infections much has been learned and the mortality reduced greatly in the last twenty-five to thirty years as a result of these investigations into the anaerobic streptococcic etiology of the disease.

Brown, (60), states that early in the disease we can consider puerperal infection as a more or less superficial wound infection and that in a portion of such cases recovery is spontaneous if drainage is sufficient. On the other hand certain of these cases have a spread of the superficial infection to the deeper tissues. The preliminary endometritis may go on to a metritis, parametritis, pelvic thrombophlebitis, pelvic abscess, pelvic peritonitis, general peritonitis, septicemia, lung infarction, and end in death. Therefore in using therapeutic measures, it is important to act in the early part of the course of the disease if at all.

The consensus of opinion in regard to the best

methods of treatment seems to be that routine bacteriological studies and cultures should be made in suspected cases; prophylaxis by avoiding trauma and damage to tissues in delivery and in examinations should be practiced. Schwarz and Dieckmann, (29), also believe that rectal examinations should be limited as they believe from experience in performing such examinations in their series of cases that if anaerobic streptococci are present in the vagina, they are more apt to contaminate the uterus by rectal examination than by direct vaginal examination of the cervix.

Schwarz and Brown, (41), advocate the use of routine vaginal instillations of mercurochrome, iodine, and glycerine similar to the procedure described by Bessesen, (62), in 1926. In their hands, this procedure led to a reduction by half of the morbidity due to puerperal infection. The later use of one per-cent neutral acriflavine in glycerin led to equally good results. In a period of eight years these authors state they have had just one fatal case of thrombophlebitis and the incidence of this lesion has been greatly reduced. They believe

their results to be striking enough to indicate that considerable value is derived from the instillations.

Polak, (63), in 1925 reported the results of a study of the use of acriflavine, mercurochrome, and gentian violet intravenously as compared with blood transfusions in puerperal infections. His conclusions were:

1. The puerpera who recovers from a puerperal infection, whether it be in the blood stream or the local tissues, does so as the result of a reaction sufficient to inhibit the further growth of the bacterial invader:
2. It has been shown experimentally and clinically that these dyes in a concentration of one to ten thousand, which is the highest concentration compatible to life, irritate the liver, heart and kidneys, and result in a definite pathology in each of these organs; that the intravenous use of these dyes will give only a temporary increase in the number of leucocytes; and that this increase is not maintained for longer than twenty-four hours;

3. On the other hand blood transfusion does increase the cellular elements of the blood; does increase its alkalinity and that of the tissue fluids and thus increases the reaction against bacteria as well as improving the function of the several eliminating organs.

Schwarz and Dieckmann, (29), feel that fewer of their cases developed thrombophlebitis because they have treated their uterine lesions promptly. In any case where there is a profuse, foul-smelling discharge, they have made it a point to remove retained secundines or clots by the digital method at the time of obtaining material for culture. In the place of the digital method a blunt curette may be used. This is followed by a dousche with one to four thousand potassium permanganate.

They feel that early in these infections the organism is rather superficial and by removing the dead material on which it can grow, much is done to prevent the spread of the infection.

In their cases of thrombophlebitis Schwarz and Dieckmann had hoped that on account of the

saprophytic nature of the organism in the non-invasive state, that the virulence of the organism might be exhausted and that by supporting the patient with frequent blood transfusions and keeping up nutrition by forced feeding, the infection would ultimately terminate favorably. Four cases were handled in this manner with only one recovery.

Ligation of the internal iliac as well as the ovarian veins, as first suggested by Bumm, was carried out in Schottmuller's clinic, (29), but the results were not gratifying perhaps because the conditions were not optimal.

The whole subject of operative treatment in puerperal thrombophlebitis and pyemia was reviewed in a paper by C. Jeff Miller, (64), in 1917. He collected one hundred ninety-seven cases from the literature. The corrected mortality, as he termed it, was thirty-three per-cent in this series.

Barton Cook Hirst in a discussion of this paper stated that before hearing Dr. Miller's results, he had believed that no advantage could be gained by this method of treatment and that the mortality had been very discouraging, (29). After hearing Dr. Miller's paper, however, the record was much more

favorable than he had expected. He stated that after a rather comprehensive study of the literature on the subject he had dismissed it from his mind, but that after hearing Dr. Miller, he felt that he would have to reconsider the subject with a mind much more open to conviction.

In 1922, Baldwin, (65), reported his results in operative treatment of puerperal infections. He reported sixty-seven cases with forty-seven recoveries. His method of treatment was hysterectomy with free drainage of the infected veins. He stated that without operative treatment, all such cases died. He further stated that in those rare cases in which the disease is limited to the ovarian veins, ligation of the veins above the thrombus is feasible, but the death rate as shown by Miller is not less than sixty per-cent. He concluded that radical operation with free drainage of all the infected veins, and usually hysterectomy, gave a death rate of less than thirty per-cent or only one half the rate of ligation and the former in a more serious class of cases.

Baldwin opined, "that all surgeons recognize the gravity of a neglected palmar abscess, or a wound received in operating or dissecting. Ear specialists



long ago learned the vital importance of cleaning out (with ligation if necessary) the thrombosed internal jugular vein in cases of lateral sinus infection due to disease of the mastoid; and there is certainly a striking similarity between an infected thrombus in the jugular and the same in the veins of the pelvis. In these and in similar conditions the 'antiphlogistic touch of the therapeutic knife' as Pancoast called it, is recognized as of absolute therapeutic importance".

Turenne, (66), in an article on "Puerperal Septic Uteropelvic Thrombophlebitis", concluded that:

1. There is a rational prophylaxis of puerperal septic thrombophlebitis.
2. Thrombophlebitis has signs, symptoms, and a clinical evolution which permit a diagnosis to be made in the majority of cases.
3. Although in more than half the cases there is a tendency toward subsidence and recovery, the high mortality justifies modern methods of treatment.
4. Surgical intervention, especially ligation of the thrombosed veins, is rational.
5. The transperitoneal route is preferable.
6. Ligation of all the efferent venous trunks

of the genital zone is desirable.

7. Resection or evacuation of the thrombus should be only exceptionally resorted to.

8. The results obtained from direct intervention on the thrombosed vein should encourage new attempts at operations to fix definitely the field of operation.

9. Operation on the veins is contraindicated in cases of permanent bacteremia, in accessible thromboses, and in cases of visceral pyemic localizations.

Schwarz and Dieckmann, (29), definitely concluded that in future cases of pelvic thrombophlebitis due to anaerobic organisms, in particular the *Streptococcus putridus*, they would attempt ligation of all pelvic veins, and if the patient's condition justified further procedure, they would remove the infected uterus with tubes and ovaries, both for the purpose of removing the infection and to limit the degree of pelvic edema which accompanies a ligation of this kind. The point and the time at which to ligate puzzled them greatly. They now feel (1927) that it should be done as soon as the organism has been recovered from the blood stream in connection with a

chill. They also feel that it is important to cleanse the uterus as previously described in all cases which have a profuse, foul discharge. In this manner, they are of the opinion that the spread of this infection to the deeper structures can be prevented.

In concluding this section on puerperal fever let it be said that:

1. It has been definitely proved by Schottmuller, White, Schwarz, Dieckmann, Brown, et al, that anaerobic streptococci can and do give rise to all grades of puerperal sepsis. The isolation of these organisms from the vaginae of forty per-cent of normal pregnant women confirms the conclusions of Rosowsky, (67), that, "these bacteria live saprophytically in the vagina, but under certain conditions following abortion or delivery they can cause severe sickness".

2. The proper incidence of various types of infection in the puerperium can only be determined by careful bacteriological studies. The making of uterine and blood cultures in cases of suspected puerperal infection, especially cases of thrombophlebitis, will give better indications as to what

cases should be ligated and as to the time at which it should be done.

3. At the completion in 1936 of a ten year study continued as a routine procedure, Schwarz and Brown, (41), confirmed all the contentions of Schottmuller and stated, "As these facts become more generally appreciated and as we gain more knowledge concerning the etiology and treatment of this type of puerperal infection, the name of Hugo Schottmuller shall take equal rank with that of the great Semmelweis".

B. Septicemias Due To Anaerobic Streptococci  
In Synergism With Bacillus Funduliformis

Lemierre, (68), describes a group of septicemias which are caused by several species of anaerobic organisms of which the anaerobic streptococcus is present in many cases. Lemierre states that all of these anaerobic organisms although they may be of different species, have in common that:

1. They live as saprophytes in the natural cavities of the human body, mouth, pharynx, intestine, and genito-urinary passages.
2. They are fragile.
3. They may be very slightly motile.
4. They grow sparsely on culture media.

These septicemias arise from inflammatory or suppurative lesions in the tissues or cavities where the above mentioned anaerobic organisms exist under physiological conditions. Having proliferated in these localities, they pass into the blood stream and frequently give rise to septic emboli in distant areas. Such septicemias tend to arise from:

1. Inflammatory lesions of the nasopharynx, particularly tonsillar and peritonsillar abscesses.

2. Similar lesions of the mouth and jaws.
3. Otitis media or mastoiditis.
4. Purulent endometritis following parturition.
5. Appendicitis.
6. Infections of the urinary passages.

Whatever their origin, these septicemias present certain common clinical aspects which enable them to be grouped together. The post-anginal septicemias are described by Lemierre, (68), as typical of the whole group.

In Germany where Schottmuller, according to Lemierre, must be given the credit for being the first to describe the post-anginal septicemias, in 1918, the importance of these septicemias has been emphasized by a number of physicians including such men as Bingold, Frankel, Claus, and Kissling. The name given by this group of men to the usual causal organism is *Bacillus symbiophiles*, and they state that it is usually associated with an anaerobic streptococcus. It is very possible that *B. symbiophiles* and *B. funduliformis* are identical. The description given by the German writers to this group of septicemias corresponds feature for feature with the observations of Lemierre.

The disease usually affects young adults or ad-

olescents equally in both sexes. Claus and Kissling have observed that sometimes small epidemics occur, a fact which is confirmed by Lemierre, (68). The most usual initial cause is a tonsillar or peritonsillar abscess which has been opened too late or not wide enough.

Since the original work of E. Frankel in 1919, German authorities have considered that these septicemias are the result of a thrombophlebitis of the tonsillar and peritonsillar veins, (68). From here the process may spread to the internal jugular or even to the facial vein. Lemierre quotes the above work and states that he has found the same to be true.

Symptoms and Course -- The first symptom of septicemia complicating the pharyngeal inflammation is a rise of temperature to 101 or 103 degrees F. An intense rigor accompanies this rise in temperature. The rigor usually begins on the fourth or fifth day following the beginning of the sore throat, occasionally as late as the eighth, tenth, or twelfth day, by which time the tonsillar infection appears to be cured and the fever has subsided. Following this, rigors occur every day several times a day or maybe at intervals of a longer time.

There is usually painful swelling of the glands below the maxillary angle usually on only one side but sometimes both. There is mild local edema and tenderness on pressure and on movement of the head. This occurs on the lateral aspects of the neck, parallel to the sternocleidomastoid muscle, and extends from the angle of the jaw to the clavicle. Suppuration may occur at this site.

Lemierre states that the septicemias that he has observed have never been pure septicemias, being always accompanied by the formation of distant metastatic abscesses. The most frequent site for the localization of such secondary abscesses has been, in his experience, the lung. These occur early and may be present from the first day. They are in the nature of septic infarcts leading nearly invariably to the formation of multiple abscesses announced by intense thoracic pain sudden in onset, dyspnoea, sometimes rusty sputum, pleural friction rub, and localized areas of subcrepitant rales. Sometimes these infarcts are accompanied by purulent pleural effusion or more rarely the effusion may be sero-fibrinous and aseptic. The purulent effusions may open into a bronchus and give rise to pyopneumothorax. Articular lesions



occur frequently, ranging from simple pains in the joints sometimes very severe, to suppurative arthritis occurring especially in the shoulders, elbows, knees, sternoclavicular or sacro-iliac joints.

Icterus and subicterus have been noted and urobilin in considerable quantities is invariably present in the urine.

Renal lesions are manifested by albuminuria, sometimes accompanied by a considerable increase in the blood urea.

Lemierre states that he has also noted thyroiditis, suppurative peritonitis, abscess in the psoas muscle or in the deep muscles of the buttock originating from sacro-iliac arthritis.

During the course of the septicemia there is usually a leucocytosis ranging from thirteen thousand to thirty thousand white cells and in the chronic cases there is a reduction in the number of the red cells to two or three million.

These septicemias may progress rapidly and end fatally in from one to two weeks. In these cases the temperature remains high constantly, the patient is in a state of extreme prostration, and dies in a state of coma. In other cases the repeated recurrence

of attacks of extreme high temperature may bring about fatal collapse in a few days. The fatal termination may be delayed to the end of the third week or even to six weeks. In these cases the temperature is irregular and oscillating. With the recurrent, excessive febrile attacks one sees the usual accompanying phenomena. Pulmonary infarcts, pleural effusions, and arthritic phenomena appear. The patient becomes cachectic; his color is pale and earthy; and he sweats profusely. Finally there is delirium and death occurs in a condition of cachexia. Death may be hastened by the sudden bursting of an abscess into a bronchus followed by syncope.

Prognosis -- This is extremely grave in these septicemias. Of twenty cases observed by Lemierre and colleagues, only two recovered. In the surviving cases the symptoms and complications were just as severe as in the fatal cases. Also the number of organisms in the blood was just as great. In the surviving cases cure occurred spontaneously with only symptomatic treatment.

Diagnosis -- This is made by means of the symptoms of septicemia as set forth above along with the signs and complications as they may occur.

Blood cultures and aspiration of abscesses clinch the diagnosis. Better results are obtained with the blood cultures if the culture is taken during a rigor. Cultures must be made on anaerobic media it must be remembered.

To anyone acquainted with the nature of these septicemias it becomes relatively easy to make a diagnosis simply on the basis of clinical findings. The appearance and repetition several days after the onset of a sore throat and particularly of a tonsillar abscess, of severe attacks of fever with an initial rigor, or still more certainly, the occurrence of pulmonary infarcts and arthritic manifestations constitute a syndrome, according to Lemierre, which is so characteristic that a mistake is practically impossible.

Treatment -- General and symptomatic treatment are the same as for any of the acute septicemias. Special treatment in cases of this character consists of ligation of the internal jugular vein on the side of the affected tonsil as soon as a positive diagnosis has been made. This has been reported with enthusiasm by many German workers. Lemierre states that unhappily he tried it in one case without success.

C. Non Tuberculous Diseases  
of the  
Bladder and Kidney

There is not much to be found on this subject in regard to anaerobic streptococci in the literature.

David, (69), in a study of such cases prompted by a desire to make bacteriological as well as clinical diagnoses of cases presumably of infection found anaerobic streptococci in association with other organisms in a notable percentage of cases.

He reviewed the literature in regard to anaerobic infections, and of especial importance in relation to the subject of bladder and kidney infections is his quotation of the report by Albarran and Cottet of nearly seventy cases of infections of the urinary tract studied by aerobic and anaerobic methods. In twenty-five cases of urinary infiltration anaerobes were isolated seven times in pure culture and eleven times in association with aerobes. In twenty-three cases of periurethral abscess anaerobes were isolated in all but three. In these forty-eight cases anaerobes were present in eighty-six per-cent and in pure culture in thirty-three per-cent.

Among the most commonly found organisms was *Micrococcus fetidus*. *Staphylococcus parvulus* was also found.

Four cases of cystitis and ten of pyonephrosis were also reported. In three cases of cystitis there was found *Diplococcus reniformis*, which is the only anaerobe described in the literature to my knowledge in relation to cystitis.

In a case of vesical tumor a small Gram-negative, obligatory anaerobic coccus occurring singly, in groups, and in chains was found. It was in symbiosis with a black pigment-forming bacillus. This coccus corresponds to no known anaerobic coccus.

D. Postoperative Progressive Bacterial  
Synergistic Gangrene

This is one of the most striking examples of chronic gangrene of the skin. It is that gangrene which occasionally follows the draining of a deep abscess in either the chest or the peritoneal cavity. There has been an increasing interest in this condition since T. S. Cullen, (70), described what he thought at the time to be the first case of this kind to be reported. This was in 1924. It has been reported with great frequency since that time and probably had been reported many times before that but was not recognized because of the fact that the titles of the papers written on the subject failed to reveal the true nature of the subject and also because of the great amount of literature on the general subject of gangrene. Since Cullen's work cases have been reported by Brewer and Meleny,(71), Alexander,(72), Shipley,(73), Gillespie,(74), Freeman,(75), Mayeda,(76), and more recently by Ballin and Morse,(77), Lynn,(78), Meleny (see Bibliography for many articles), Baker and Terry,(80), Horsley,(81), Carol,(82), Poate,(83), Patterson,(84), Christopher,

(85), and Probststein and Seelig,(86). A few cases have been reported which developed spontaneously, (Lockett - 87).

Etiology -- In most of the cases reported only routine bacteriological studies have been made. A variety of organisms has been reported as a result of the efforts of these authors to determine the bacterial cause of this lesion. None of these organisms seems to be of any special significance in this respect. Brewer and Meleny,(71), and Meleny alone,(79), have utilized special methods in the bacteriological study of several cases and since the findings were identical they believe them to be of significance. Cultures were made from all parts of the lesions including the gangrenous tissue as well as the spreading periphery. Micro-aerophilic streptococci were found in pure culture at the periphery of the lesions not only in the reddened, obviously involved tissue, but also in the more peripheral apparently normal tissue. In the frankly gangrenous tissue the micro-aerophilic streptococcus was found in company with other organisms, a hemolytic *Staph. aureus* and a diphtheroid bacillus. The diphtheroid bacillus was entirely non-pathogenic for

animals when injected either in pure culture or together with either of the other organisms. When the streptococcus or the staphylococcus were injected in pure culture, no lesion was produced in animals, but when the two were combined in equal doses and injected, a gangrenous lesion resulted very similar to those observed in humans. From these observations arose the theory that the process involved in these gangrenous lesions was one of synergism between these two organisms and that the streptococcus went ahead into the normal tissue and paved the way, so to speak, for the gangrenous activity of the two organisms combined.

The streptococcus in this lesion is not a strict anaerobe. It will grow aerobically after a number of transplants in artificial media with a reduced oxygen tension. However, anaerobic methods are necessary to obtain a culture of the organism from the periphery of a freshly excised lesion in the red zone. This streptococcus is the same as *S. evolutus* of Prevot, (34).

The source of infection is presumed to be from the intestinal tract in the abdominal cases since this organism is commonly found in this location.



In the chest cases the source of infection seems to be from the mouth or from emboli released from thrombosed vessels in the peritoneum.

Symptomatology -- In the most of the reported cases this lesion has followed the surgical drainage of peritoneal abscesses. Most often it makes its initial appearance about a week or two following the operation as an infection of the whole wound or as an area of induration around retention sutures. In the beginning the wound is seen to become reddened, swollen, and tender. In a few days the wound margins and/or stitches assume an appearance like that of a carbuncle. The center of the involved area becomes purplish and the periphery becomes a brilliant red color. Now comes the salient feature of the disease, an exquisite tenderness. Within a few days the purple colored areas become a dirty grayish brown and dull in appearance and these areas are seen to consist of dead skin and to be frankly gangrenous. The red zone gives way to the advancing purplish zone and as this progresses peripherally the skin becomes swollen and stands up above the level of the surrounding areas. The central margin of the purple zone toward the gangrene is well defined but on the periphery the purple

zone fades off gradually into the red zone which latter slowly advances farther to the periphery. There is very little undermining of the normal skin and the purple zone is firmly adherent to the gangrenous skin. The width of the involved circle of skin remains fairly constant although the circumference of the whole lesion increases. This, as Meleny explains it, (88), is due to the fact that as the entire process progresses and the zones migrate peripherally, the central area of gangrenous skin liquefies at its central margins leaving in the center of an irregularly doughnut-shaped lesion, an area of tissue uncovered by skin.

As this central area of necrotic skin becomes liquefied and sloughs off, there is left a base of granulation tissue which gradually enlarges. The destruction of the skin may not be complete and here and there patches of dermis from hair follicles and sweat glands may be left giving rise to regeneration, in patches, of epithelium. Although as the process proceeds over a long time the patient may become discouraged and worn down with pain, there is usually very little general reaction such as

fever or anemia and the patient remains in fairly good general condition. In the past, often the true nature of the disease has not been recognized until there has been quite extensive destruction of skin and subcutaneous tissue. Earlier diagnosis by signs and symptoms together with adequate bacteriological studies should remedy this situation since there is now a good method of treatment for this particular lesion.

Diagnosis -- This is made by the clinical onset, signs and symptoms as portrayed above, and by adequate bacteriological studies as are also described above. For methods of anerobic culture see the section of this thesis on culture methods included under Bacteriology, page 58. Confusion and poor results have been the outcome of bacterial studies being made too late and frequently not the proper culture methods have been used. This is important because the treatment of the various groups differs markedly and delay in diagnosis results in delay in instituting proper therapy. Anaerobic as well as aerobic methods should be used and culture media used which are suitable for any of the organisms which may be present.

Studies should be made early and cultures taken from various parts of the lesion in particular the zone of advance.

Differential Diagnosis -- This type of gangrene is easily differentiated from that type due to the gas gangrene group of organisms and the hemolytic streptococci because of the acute nature of the latter. A diagnosis by symptoms and signs is of a special importance in the acute gangrenes because of the need for early institution of proper treatment. In the anaerobic streptococcic type of gangrene which is characterized by chronicity there is usually sufficient time for a thorough bacteriological study of cultures properly taken from the lesion. For this reason a bacteriological diagnosis assumes greater importance in these cases. Also, although all four types of chronic gangrene have usually certain distinguishing clinical signs or symptoms, this is not always the case and bacteriological studies are often essential. However, many of these cases are treated in the home, office, or hospitals not equipped for extensive bacteriological studies and under these conditions diagnosis

Fusospirochaetal gangrene.	Essential organisms fusiform bacilli, spirilla and spirochaete usually associated with non-hemolytic streptococci.	Usually occurs in a wound contaminated with mouth secretions, e.g. human bite. Early inflammation with a gradual development of necrosis of wound edges with penetration to bones and joints.	Extensive sloughing of superficial and deep tissue with multiple sinus formation. Organisms profuse in exudate and in the necrotic tissues.	Intensive intravenous administration of neoarsphenamine. Radical amputation if medication fails.
Amoebic infection with gangrene	Essential organism <i>Entamoeba histolytica</i> associated with numerous streptococci, staphylococci, and fecal organisms.	Usually follows the drainage of an amoebic abscess of the liver. Margins raised and everted. Granulations have appearance of raw beef covered with shreds of necrotic material. Clairy pus expressed from margin.	Extensive destruction of dermis with undermining and secondary destruction of epidermis. Polymorphonuclear exudation. Amoebae and bacteria numerous in the exudate and in the tissues.	Intensive administration of emetin hydrochloride intravenously. Radical excision of the lesion if disease is limited to the skin and medication fails.

Treatment -- Prophylaxis consists of leaving operative wounds unsutured when peritoneal abscesses, lung abscesses, or empyemas have been drained. This prevents tissue tension which favors the establishment of the disease in the presence of contaminated wounds such as exist in this type of surgery.

Conservative methods of active treatment have in every case proved fruitless. Meleny in 1933 advises radical excision of the entire lesion, including the outer zone of redness, immediately. This excision is equally and greatly successful with either the knife or the radio-knife. Cautery is not so desirable because there is more destruction of tissue and healing must wait on the separation of this destroyed tissue from the good.

More recently Meleny, (79, 88, 89, 91, 92, 94), Meleny and Harvey, (90), Meleny and Hohnson, (93), Reicher, (95), Brewer and Meleny, (71), Shallow, (96), Pennoyer, (97), Lawrence, (100), and others (see following section) have reported favorably on the use of zinc peroxide and sulfanilamide in the treatment of this and similar conditions.

E. Chronic Ulcerative Burrowing Non-Gangrenous  
Ulcer Due to Micro-aerophilic Hemolytic Streptococci

"Initiated by the extensive work of Meleny, (89), and associates a group of chronic ulcers has received an etiological and therapeutic elucidation which appears to have furnished medicine with a new disease and a new therapeutic agent, zinc peroxide" -- (95). Meleny calls this condition, "chronic, ulcerative, burrowing, non-gangrenous ulcer due to micro-aerophilic hemolytic streptococci." He emphasizes both the anaerobic and hemolytic characters of the organism as well as either or both undermining and burrowing tendencies of the lesion.

From some brief case abstracts in an article, (89), by Meleny in 1935, may be drawn a composite picture of this condition:

"The disease begins gradually. What appears to be an ordinary drainage tract from an abscess of deep or subcutaneous nature fails to heal in the usual manner. Any improvement which may have been present earlier gradually ceases. There is liquefaction of the subcutaneous fat and connective tissue with undermining of the skin margins. There

is no gangrene, but the skin edges roll in. Undermining progresses and there is widening of the opening in the skin. A base of gelatinous, pale, granulation tissue is exposed. In places in the area surrounding the lesion the skin may assume a dull red or bluish appearance. The undermining has extended beneath this area and the skin has thinned out as if it were being liquefied from beneath. After some weeks time an opening appears in this thinned skin. This secondary opening gradually enlarges, and its skin edges may extend until they have fused with the margins of the original lesion."

In lower abdominal lesions there is often a spreading of the undermining down toward the groin or pubic region extending into the vulva or scrotum or into the thigh. It may extend inward into the pelvis, by dissecting through the muscles, and give rise to deep sinuses. The undermining is prone to stop at places where skin is firmly attached to underlying tissue such as the umbilical area or at the crest of the ilium. There may be areas around the margin of the lesion where undermining does not occur and here healing takes place by new growth



of epithelium. Instead of progressing steadily, the margin of new skin may become clear-cut and remain stationary for a long time and may even rapidly melt away. The disease may show gradual improvement from day to day for a long time and then suddenly all the advantage that was gained is lost and the disease progresses more rapidly than ever.

Etiology: - the essential organism is an hemolytic streptococcus which prefers an anaerobic environment. Its immediate source is probably either the intestinal tract or the vagina. Often it can be obtained only by anaerobic cultivation and is sometimes present in pure culture. It is sometimes found with aerobic cultivation but even so it grows better anaerobically. After artificial cultivation on meat medium it takes on aerobic properties and will grow on an aerobic plate after a few generations. The colonies are small and gray and surrounded by a clear hemolytic zone with a diameter from two to three times that of the colony. It shows the usual cultural characteristics of beta hemolytic streptococci and Meleny thinks it may have been at one time an ordinary aerobe which has adapted itself

to the anaerobic environment of the intestinal tract.

Colebrook, (30), may have cultured such an organism but if so, he did not distinguish it from other hemolytic streptococci. Smith, (101)), probably found it in cases of lung abscess according to Meleny. Thompson, (102), did not mention this organism in his report of the role of streptococci in skin diseases.

This organism belongs to the group of "anaerobes by predilection" of Prevot, (34), though according to Meleny he did not mention any hemolytic characters.

This organism has probably been present frequently in such infections, but has been unrecognized because of the fact that anaerobic methods have not been used. On occasions when this organism has been cultured aerobically from the surface of such a lesion it has probably been thought to be an ordinary hemolytic streptococcus. According to Meleny, (89), it can only be identified by comparison of both aerobic and anaerobic cultures. Meleny prefers the term micro-aerophilic to describe this organism.

Symptoms and Signs: - there is usually only moderate pain, but it may be severe. Daily

temperatures to 101 - 103 degrees F. occur. During the febrile period the patient is usually prostrated. Lack of response to treatment over a long period may break the patient's morale to the point of suicidal attempts. After months or years, the disease may heal spontaneously or produce death by burrowing and erosion of a great vessel or by amyloid degeneration of liver, spleen, and kidneys.

Treatment: - various writers have suggested various modifications of treatment for this group of infections. Meleny applied zinc peroxide in distilled water, Pennoyer, (97), utilized hydrogen peroxide in which to suspend the zinc peroxide. Rhoads, (99), believes it to be of value to give zinc peroxide suspension in the form of a low rectal enema in cases where a perirectal abscess opens a short distance proximal to the anus. Mullen, (98), and Lawrence, (100), both treated these infections with zinc peroxide and sulfanilamide together. Meleny tried similar treatment in a large series of cases, (90). Shallow, (96), tried Prontosil without success in one case. He did not state the dosage used, however. Meleny used

sulfanilamide alone in one case. This treatment was considered successful, but the course of the infection was longer than in those cases treated by the combined method. Goodman, (103), however, had good results with sulfanilamide alone in the treatment of small, chronic, undermining ulcers. He made no anaerobic studies, though. Ayres, (104), was met with final success in the treatment of ulcerations which resembled clinically those under consideration; he used maggots. Meleny, (89), has used zinc peroxide in many cases of chronic infectious gangrene and chronic, undermining, burrowing ulcer due to anaerobic streptococci. He has had good results with this method of treatment in the great majority of these cases. He reports that the zinc peroxide has changed favorably the course of the disease and resulted in rapid resolution. Meleny states that, "in no case has there been evidence of irritation of tissues by the zinc peroxide; granulations have not been disturbed; healing has been favored and foul odors have disappeared".

Within the last six months there have been in the University Hospital of the University of Nebraska at Omaha two cases of the nature of those under dis-

cussion. Following are brief case reports of these two cases:

1. Case #66214 - Mrs. R. C., a married woman aged 35, entered the hospital 12-19-39 with an infected ulcerating lesion of the right thigh. The patient stated that she first noticed this sore on her leg in July of 1939 following some mosquito bites which she scratched. The lesions first appeared as abscesses which later broke down and became ulcers. New lesions developed in the area surrounding the original lesion. These tended to coalesce and merge with lesions already present. There was an area of inflammation around the lesions and pus was discharged. There was no evidence of healing. The lesions were found to be undermining so wide surgical drainage was performed and the lesions were packed with zinc peroxide packs. The patient was first seen by the department of dermatology where it was at first thought the condition might be on a luetic basis, but subsequent Kahn, Kline, and Wasserman tests discouraged this diagnosis. Later several cultures were made from the lesions and anaerobic streptococci were found on almost every occasion in company with other organisms.

This patient had a very stormy course during her three hundred forty-eight day stay in the hospital. At times the lesions seemed to be healing well only to again break down and start undermining. On 8-28-40 ultraviolet radiation was started. The results of this were questionable. On 9-3-40 she was started on irrigations with one to five thousand potassium permanganate solution followed each time by sprinkling the wound with sulfanilamide powder. Under this regime there was manifested remarkable healing. Because the sulfanilamide tended to form crusts under which the infection tended to spread, dressings of 1% sulfathiazole in cod liver oil were substituted with continued improvement of the wound.

2. Case #702263 - E. M., schoolboy aged 15, entered the hospital 1-24-41 with a swelling mass in the right cheek and inability to open his mouth more than about a quarter of the normal distance. The patient related that he had had his tonsils removed on August 15, 1940 and that on about August 25, 1940 he noticed a swelling in front of the right ear lobe. He had a tooth pulled in an attempt to relieve the swelling but this was to no avail. The mass has continued to enlarge since then.

On examination the mass was firm and fixed. It was not painful but slightly tender to touch. The patient was only able to open his jaws a very short distance. The tumor showed no enlargement with salivation. First impressions were: 1. Osteosarcoma of jaw. 2. Fibrosarcoma of jaw. 3. Mixed tumor of parotid or parotid cyst.

A biopsy was performed and an abscess was opened. Three days later the face was markedly swollen so the sutures were removed and a large amount of thick, green-gray pus with a fecal odor escaped. Cultures of the lesion revealed anaerobic streptococci and diphtheroids living in symbiosis. Surgical drainage and a short course of sulfathiazole (gr. XV qid for three days) met with prompt results in this case.

Reicher,(95), believes that some modification in certain of the accepted concepts regarding this disease is justified by certain features noted in some of his cases. One case was typical and fit precisely in with this group of infections; the ulcer was chronic, exhibited undermining and burrowing, was caused by the anaerobic, hemolytic streptococcus and responded dramatically to zinc peroxide treatment. Another case exhibited undermining and burrowing and responded nicely to zinc peroxide but

was associated with a non-hemolytic anaerobic streptococcus; this case illustrates that the clinical picture described by Meleny can be duplicated by a different type of anaerobic streptococcus. Reicher's results in the treatment of these cases were "such as to lend unqualified confirmation of the effectiveness" of zinc peroxide as a therapeutic agent. Two cases in this series illustrate the fact that the significant anaerobic organism present in either of the above described cases may be isolated in large numbers from lesions which do not undermine or burrow. In these types cure may be obtained by the use of simpler local antiseptics combined with short and possible insignificant courses of sulfanilamide.

Reicher concluded:

1. It appears that hemolysis or non-hemolysis is not essential in producing the gross picture of the disease so long as the streptococcus is anaerobic.
2. The anaerobic streptococcus may invade without causing undermining or burrowing.
3. Treatment should depend on an evaluation



of the combined factors of causative organism and character of lesion.

Based on the foregoing, the following corollaries may be presented:

1. Anaerobic as well as aerobic culture studies should be made in all superficial infections, especially if they tend to chronicity.
2. If the infection is of recent origin, not extensive and not burrowing in nature, treatment with the more conventional antiseptics should be instituted regardless of the type of organism isolated.
3. A burrowing or undermining, extensive or stubborn lesion associated with an anaerobic streptococcus (hemolytic or not) should be treated according to Meleny's principles, namely, immediate application of zinc peroxide and oral sulfanilamide (gr. 18 q 4-6 hrs.).
4. A careful watch should be maintained throughout the course of the ulceration for the appearance of any new areas of undermining or burrowing.

## SUMMARY

The history of anaerobic streptococci has been reviewed from 1884, which is the earliest date I have been able to find in connection with these organisms to the present. The early work on this subject is reported in the French literature and followed shortly in that of the Germans. Only more recently has much been done in this country regarding this group of bacteria.

The Bacteriology of the anaerobic streptococci has been reviewed to some length, including classification, differentiation, habitat and pathogenic activities, anaerobic culture methods, and criteria of anaerobiosis.

The clinical aspects of this subject have been quite thoroughly reviewed and the relation of the anaerobic streptococci to puerperal sepsis, septicemias, diseases of the kidneys and bladder, and a group of chronic infections diseases of the skin and subcutaneous tissues has been surveyed and found to be one of extreme importance and well worthy of the effort put forth in such a study.

## CONCLUSIONS

That anaerobic streptococci bear an etiological relationship to human disease has been recognized since the time of Lemaistre in 1886.

Anaerobic streptococci are of widespread importance as a causative factor in many diseases, especially those of chronic, putrid suppurative, and gangrenous nature.

There are many species of anaerobic streptococci; these may be roughly classified and differentiated; many of them have been shown to be pathogenic to animals and to men.

There are simple and practical methods of culture for the aid of the study of these organisms and for the purpose of diagnosis.

There are in many instances valuable specific methods of treating diseases caused by the anaerobic streptococci and these methods differ from and are more efficacious than certain time-worn remedies for lesions categorically placed in certain groups on a clinical basis alone. Zinc peroxide, sulfanilamide, and as demonstrated in the cases here at the University Hospital, sulfathiazole, are of value in

the treatment of certain gangrenous or ulcerating lesions of the skin and subcutaneous tissue of a chronic nature. The prophylactic and active measures for combatting puerperal infections caused by the anaerobic streptococci as described under that heading in this paper have done much to greatly decrease the mortality in that disease. All of these measures should be included as valuable additions to the armamentarium of the modern physician in view of the findings here presented as regards the anaerobic streptococcic diseases.

By correlating the pathological and anatomical diagnosis with the diagnosis based upon bacteriological study of the causative organism in an infectious disease, we can hope to better our results in the treatment of these diseases by fitting our therapeutic agent to the work it must do as well as to the conditions under which it must do it. Therefore, in particular, I wish to emphasize that anaerobic culture methods should be included as a measure of routine in all cases where bacteriological study is required as an aid to diagnosis. In this way the clinician will be rewarded in a worthwhile percentage of cases by positive findings where none would have been pres-

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