

THE ANAEROBIC BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT IN CHILDREN

A THESIS

bу

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INTRODUCTION

During two years spent as a technician in the Department of Bacteriology of the Children's Memorial Hospital, it was noted how frequently cultures from children with upper respiratory tract infections yielded none of the common pathogens by routine laboratory aerobic culture methods. The etiologic agents of cases such as these might well be viral or aerobic bacteria not ordinarily recognized as pathogenic, or anaerobic bacterial species.

The anaerobic bacterial flora of the upper respiratory tract has been but little studied, possibly due to the time consuming and cumbersome methods of obtaining anaerobiosis; possibly because relatively little is known about anaerobic bacterial species, and their classification is still in a state of confusion.

It was thought, therefore, that a survey of the anaerobic flora would prove of interest, and perhaps yield further results showing some correlation between certain anaerobic species and disease, the flora of the respiratory tract at different ages, and cast a little more light upon the taxonomy of anaerobic bacteria.

This thesis presents a preliminary study.

SECTION A.

REVIEW OF THE LITERATURE

PART I GENERAL SURVEYS

Past surveys of the anaerobic bacterial flora of the upper respiratory tract have been, for the most part, incidental to the search for the etiological agent of the common cold. Very few have been made in children. was found that filtered nasopharyngeal washings taken from influenza patients could transmit the disease to healthy humans, it was thought that the primary agents of influenza could be filterable forms of bacteria. The first investigators to report the isolation of an organism from these filtered nasopharyngeal washings were Olitsky and Gates, in 1920 (70). They named their minute Gram negative bacillus Bacterium pneumosintes. Subsequent studies by these and other workers (22, 33, 34, 46, 65, 69, 71, 72, 73a,b,c,d, 74a,b, 75, 76, 77, 78) resulted in the isolation of several other similar types of filter-passing organisms from subjects in health or suffering from head colds. The conclusion was reached that these organisms constituted part of the so-called normal flora of the upper respiratory tract (61).

In the same period, the presence of a variety of anaerobic organisms in the pharyngeal cavity was noted by other investigators. Hall (35) reported having found a micrococcus, but concluded that, on the whole, anaerobes were a rare occurrence in the upper respiratory tract. Studies on influenza pursued by Branham (15) in 1927 resulted in the isolation of

seventeen strains of anaerobic microorganisms including a small Gram negative coccus, a spirochaete, several groups of Gram negative bacilli, a minute Gram positive organism of undetermined morphology and a Gram negative haemolytic diplococcus. The following year Noble and Brainard (67) conducted a survey in adults of the flora prevalent in health and during head colds. They isolated anaerobic Gram positive and Gram negative cocci and four species of Gram negative bacilli. In a second study (68) they found, in addition, an organism which they tentatively identified as Micrococcus gazogenes.

Interest in studies of this type waned when the conclusion was reached that the etiological agent of the common cold must be a filterable virus (60).

One of the earliest surveys of the anaerobic bacterial inhabitants of the oral cavity was that undertaken by Mme. Brailovsky-Lounkevitch in 1915 (14). She studied the mouth flora in infants, children and adults, and found that, in infants, the flora was almost entirely aerobic or microaerophilic, and that as teeth appeared the anaerobes increased in numbers. The organisms she reported as Leptothrix buccalis, parvulus, B. anaerobius-gracilis and Spirochaetes, Spirilla and Vibrio. A quantitative examination of the anaerobic flora under different dietary conditions was carried out by Howitt and Fleming (49) who reported the presence of Micrococcus gazogenes, Leptotrichia buccalis, fusiform bacilli and short Gram positive bacilli.

David T. Smith, in his textbook "Oral Spirochaetes

and Related Organisms in Fuso-Spirochaetal Disease" (98) listed the common mouth organisms as spirochaetes, fusiform bacilli, spirilla, vibrios and the species Bacillus gonidia-formans, Vibriothrix tonsillaris, Leptothrix buccalis, Bacterium melaninogenicum and Micrococcus gazogenes.

It was the opinion of Bibby (7) who was interested mainly in the actual number and location of oral flora that cultural studies alone failed to give a true picture. He felt that this was due to what he called the inherent selectivity of the medium. As a result, he limited his studies to morphological examinations by direct smears. Using a direct counting method he estimated the percentages of different morphological groups of organisms occurring in different locations in the mouth (8) and at different times from the same location (9). He noted the presence of fusiform bacilli, Gram positive and negative cocci, bacilli and filaments. Variations in flora from time to time, and from different locations were wide and he could establish no significant relationship between oral flora and oral health.

A similar morphological study was also undertaken by Brooke (18) using a special staining method and reporting Gram positive and negative bacilli, Gram positive and negative cocci, Gram positive and negative coccobacilli, Gram positive and negative diplococci, and streptococci, spirochaetes, fusiform bacilli and Actinomycetes.

Unfortunately, studies of this kind give no information as to whether the flora is aerobic or anaerobic.

The greater part of the literature on the buccal-

pharyngeal cavity contains reports of investigations of single groups of organisms, or pathological processes such as Vincent's infection and dental caries, and numerous accounts of single species isolated from time to time from a variety of conditions. These will be discussed under separate headings.

PART II DISCUSSION AND CLASSIFICATION OF THE ANAEROBIC COCCI

The development of the present classification of cocci has been long and complicated but there are some note-worthy steps in this development which have influenced the nomenclature of anaerobic species. Prevot (86) has criticized the American system of classification for what he considered lack of clarity and too evident an attempt to follow a system of nomenclature in a classification still under discussion. The Nitrobacteriaceae were at the basis of the American classification but Prevot believed the Coccaceae should be, as they are morphologically the most simple.

His classification follows:

	Class	Subclass	
Eubacteriales		Non-sporing Eubacte	eriales
Order	Family	Tribe	Genus
Coccaceae)	Neisseriaceae	\ Neisseria	Neisseria
) Veillonella	Veillonella
	Micrococcaceae) Streptococceae	Diplococcus
		}	Streptococcus
) Micrococceae) Gaffkya
		:) Sarcina
) Staphylococcus
) Micrococcus

Although the Veillonella were discovered in 1898 no name was given them until the time of Prevot's study, in which he excluded the genus Leuconostoc from the tribe Streptococceae and the genus Rhodococcus from Micrococceae. In 1940 Prevot's classification was revised somewhat (6) with the addition of the tribe Staphylococceae to the Family Micrococcaceae.

Family Tribe Genus

Micrococcaee Streptococcae Diplococcus, Streptococcus

Staphylococcae Gaffkya, Staphylococcus

Micrococcae Sarcina, Micrococcus

His classification is not generally accepted today. The American classification has been revised and appears as it is found in Bergey's Manual of Determinative Bacteriology, Sixth Edition (6).

Class Order Family Genus
Schizomycetes Eubacteriales Neisseriaceae Neisseria, Veillonella
Micrococcaceae Micrococcus, Gaffkya, Sarcina
Lactobacteriaceae Streptococcus, Diplococcus,
Leuconostoc

As is seen, the genera Diplococcus, Streptococcus and Leuconostoc are included in the tribe Streptococceae of the family Lactobacteriaceae. The editors of the Manual point out that the anaerobic diplococci and streptococci, many of which are putrefactive and gas-forming, seem so different from the fermentative microaerophilic diplococci, streptococci,

leuconostoc and lactobacilli that they should be separated from the Lactobacteriaceae and placed in genera and family of their own. They do not agree with Prevot who preferred to solve the difficulty by including the fermentative diplococci and streptococci in the family Micrococcaceae because of morphological resemblances which they do not consider to be fundamental.

Further investigation may lead to a more satisfactory classification, but Bergey's Manual remains the only generally accepted basis for systematic study of species.

(a) Micrococcus

Anaerobic members of the genus Micrococcus have been known for a long time, but except for Prevot's work seem to have been little studied. He isolated and studied several species from focal infections and appendicitis. Topley and Wilson (102) list Micrococcus buccalis, which was originally isolated from the mouth. This is a small, non-motile Gram positive coccus, which produces filiform growth and gas in a glucose agar stab and forms a greyish sediment and turbidity in dextrose broth. Gelatine is not liquefied, hydrogen sulphide is produced, no indole is formed. The strain ferments many carbohydrates forming gas.

Foubert and Douglas (32) have recently attempted to identify Micrococci recovered from the tonsils, uterus and skin. They list several species, one of which, Micrococcus lactilyticus is generally considered to be the species V. gazogenes belonging to the Veillonella genus and will be discussed under that heading. The other species include

M. niger, M. asaccharolyticus, M. grigoroffi, M. anaerobius, M. activus, M. prevotti, M. saccharolyticus and M. variabilis. The validity of the first four has been established and further work may determine the acceptability of the other species. The key of differentiation of these species is given below.

- A. Visible gas from peptones
 - 1) Ferments lactate

M. Lactilyticus

- 2) Does not ferment lactate
 - a) Colonies in deep agar pigmented black M. niger
 - b) Colonies not pigmented
 - 1. Liquefies gelatine

M. activus

- 2. Does not liquefy gelatine
 - (a) Growth enhanced by glucose

Strain not named

- (b) Growth not enhanced by glucose
 - (1) Indole /, potassium nitrate) M.asaccharolyticus) reduced, gas.) M. aerogenes
 - (2) Indole -, potassium nitrate-, M. prevotti gas slight.
- B. No visible gas from peptones
 - 1) Ferments glucose
 - a) Ferments lactose

M. grigoroffi

b) Does not ferment lactose

M.saccharolyticus

- 2) None or only slight fermentation of glucose
 - a) Gelatine not liquefied, cell size uniform M. anaerobius
 - b) Gelatine liquefied, cell size variable M. variabilis

The occurrence of anaerobic Micrococci in the nasopharynx has been well established. Those which have been isolated from that locality are \underline{M} . aerogenes and \underline{M} . anaerobius.

M. aerogenes (6, 86) was originally isolated from cases of puerperal fever and more recently from infected tonsils by Prevot. It is in the form of spheres from 0.6 to 0.8 micron, in clusters, occasionally pairs and short chains. Colonies are small, lenticular and nearly spherical in deep agar and very small, greyish, surrounded by a narrow clear zone on blood agar. It does not liquefy gelatine, changes neutral red serum agar to greenish yellow with the liberation of gas, and produces indole and gas in peptone water containing serum. Proteins are not attacked, the organism does not plasmolyse readily and glucose is fermented.

M. anaerobius (6, 86) was first isolated by Prevot from pus obtained from a suppurating tonsil. It is a small coccus from 0.5 to 0.6 micron. Colonies in deep agar are thick and lenticular. The species does not liquefy gelatine, does not change neutral red, forms no gas in peptone water and does not plasmolyse. It does not attack proteins.

Isolation of Micrococci

medium (114). Foubert and Douglas (32) found that the addition of blood or serum had no effect on the number and types of Micrococci isolated. These workers used a basic medium of peptone, yeast extract, glucose, sodium thioglycollate, methylene blue and water, modifying it for biochemical tests.

(b) Gaffkya, Sarcina

Gaffkya and Sarcina are probably widely distributed in nature, but no record has been found describing their

isolation from the upper respiratory tract. As they are found in stomach contents (6) they might be suspected of inhabiting the respiratory tract. However, Foubert and Douglas (32) prefer to call all strains isolated from there Micrococcus, as there is such a gradual transition between strains forming tetrads occasionally and those forming them constantly.

(c) Neisseria

The earliest member of the anaerobic Neisseria to be isolated was described as a Micrococcus or <u>Diplococcus</u> reniformis in 1899 (6). Since then two species have been described, one of which, <u>N. discoides</u> (6) has been recovered from the upper respiratory tract. Prevot (86) isolated this small Gram negative coccus from bronchial mucous. The organism has the peculiar character of growing only as disc-shaped colonies in what Prevot called the "critical zone" of anaerobiosis in deep gelatine culture. Gas is formed around the colonies. In fluid media there is turbidity and a granular deposit together with a slightly sour odour. The gas produced is inflammable. Neither indole nor hydrogen sulphide is formed, and milk, gelatine, proteins and carbohydrates are not attacked.

(d) Veillonella

The naming of species of Veillonella has been a subject of some confusion. In 1901 Lewcowicz described a small Gram negative coccus-shaped organism which he named Micrococcus gazogenes alcalescens anaerobius. Hall and

Howitt in 1925 (39) reported the isolation of this same organism from almost every human saliva examined, naming it Micrococcus gazogenes. Prevot (86) renamed it Veillonella, placing it in a new genus because of its negative reaction to the Gram stain. The species name gazogenes was considered incorrect for a Micrococcus by the editors of Bergey's Manual in 1939 and it was classified as Veillonella gazogenes in 1948. Foubert and Douglas (32) claimed that this organism appeared Gram positive in the first few hours of growth and consequently returned it to the genus Micrococcus, but suggested that the species name lactilyticus be applied to it.

Another small Gram negative anaerobic coccus was described in 1898 by Veillon and Zuber (114) which was named Staphylococcus parvulus. Some authors considered this species identical with <u>V. gazogenes</u> (15) but other workers found them to have distinctive characteristics (39) and they are now considered to be two distinct species of the genus Veillonella.

There may be other Veillonella which have not been classified. One has been isolated from the nasopharynx which is different from both of these (16).

Veillonella gazogenes (6) has been isolated from the nasopharynx from persons in health or with head colds (68). Morphologically it occurs in the form of spheres from 0.3 to 0.7 microns, in irregular masses, rarely in pairs, short chains or singly. Surface colonies on blood agar are not haemolytic. Colonies in deep agar are punctiform becoming lenticular. Gas bubbles form in sixteen to eighteen hours. The species does not produce hydrogen sulphide nor indole, fails to reduce

nitrates, does not develop foetid odours and does not ferment carbohydrates.

Prevot (86) isolated a similar organism in culture from the gums. This coccus differs from <u>V. gazogenes</u> in its ability to grow at lower temperatures. It has been named <u>V. gazogenes var. gingivalis</u>.

Micrococcus minutissimus, isolated by Oliver and Wherry (79) from mucous membranes is considered to be var.

minutissima of V. gazogenes. It differs only in that carbohydrates favour its growth and that the gas produced is not inflammable (6).

A third variety has been isolated from the mouth. This is <u>V. gazogenes var. syzgios</u>. It differs from <u>V. gazogenes</u> in the formation of small amounts of hydrogen sulphide and the reduction of nitrates by some strains (6).

Veillonella parvula, (6, 86) a small Gram negative coccus, produces colonies on blood agar which are usually surrounded by a zone of incomplete haemolysis. The colonies in Veillon's semisolid medium are punctiform becoming lenticular. Gas bubbles form, Litmus milk is neither acidified nor coagulated, small amounts of indole are formed, nitrates are reduced, hydrogen sulphide is produced but ammonia is not formed. Coagulated protein is not attacked, but acid and gas are produced in glucose, and a small amount of acid in fructose, galactose and sucrose.

Two varieties are noted. One is <u>V. parvula, var.</u>

<u>branhami</u> (6) which Branham isolated from nasal washings in

influenza (15). It is serologically distinct from <u>V. parvula</u>.

V. parvula var. thomsonii (6) differs in that it requires some accessory growth factors found in serum or similar body fluids. This organism was recovered from throat cultures made on patients with measles or scarlet fever.

Isolation of Veillonella

The isolation of these Veillonella should not be too difficult, for they are abundant in the mouth, at least in adults (67) and do not require highly specialized media for growth. However, they stain so poorly by Gram's method and are so minute that they may be almost invisible in pure culture and overlooked in mixed ones. Hall and Howitt (39) suggested the isolation of <u>V. gazogenes</u> in deep meat infusion peptone agar inoculated with serial dilutions of brain broth cultures. Colonies were distinctive in having a so-called buckwheat appearance, and a pure culture yielded abundant gas.

(e) Diplococcus

There are five recognized anaerobic species of Diplococcus. Three of these are of primary interest as they have been recovered from the upper respiratory tract.

D. paleopneumoniae (6, 86) was originally isolated by Rist from the nasopharyngeal cavity. Morphologically it has been described as encapsulated Gram positive spheres of 0.7 to 1.0 microns occurring in pairs. The organism forms round, raised transparent colonies on agar. Development is rapid and abundant on blood agar, and good growth is obtained in broth which is in the form of opalescent turbidity settling

out as a flocculent precipitate. Growth is good in Litmus milk, the medium being partially coagulated, but poor in peptone water. Gelatine is not liquefied. Acid but no gas is produced from glucose and lactose.

Tunnicliff isolated an anaerobic diplococcus from mucous secretions and blood cultures from cases of measles (103). This she named <u>D. rubeolae</u>. The name was later withdrawn (110) and that of <u>D. morbillorum</u> substituted. <u>D. morbillorum</u> (6, 86) requires serum or ascitic fluid for its growth. It is in the form of Gram positive spheres from 0.6 to 0.8 microns in short chains, rarely in small masses. It forms small punctiform colonies on serum agar. Blood agar colonies are surrounded by a greenish halo and may be quite large. Gelatine is not liquefied, Litmus milk is not changed by most strains, indole is not formed. The species is not soluble in bile, and forms acid from glucose, sucrose and maltose.

D. constellatus (6, 86) has been isolated from the throat in a case of tonsillitis. The spheres are from 0.5 to 0.6 microns, occurring in pairs or tetrads. Deep agar colonies are at first very small but as they become larger are surrounded by many satellite colonies. Growth is poor in plain broth, good in glucose or blood broth. Growth is good on gelatine but the medium is not liquefied. Proteins are not attacked; neutral red broth is unchanged. No gas nor odour is produced, but acid is formed from glucose and arabinose.

(f) Streptococcus

An anaerobic streptococcus was first described by

Veillon in 1893. He isolated Micrococcus foetidus from material from cases of Ludwig's angina, perinephric abscess and bartholinitis. In a review of the status of the anaerobes of this genus up to 1937, McDonald, Henthorne and Thompson (64) reported that these organisms have been found in a variety of localities including the vagina, lochia, infected wounds and appendical abscesses. It had been thought that all strains belonged to a single species, Streptococcus anaerobius micros, but many workers were able to describe distinctly different strains. McDonald and co-workers quoted and agreed with Taylor's conclusion that classification of this group of organisms on the basis of carbohydrate fermentation characters was impossible. These authors therefore, attempted a serological classification Numerous other investigators have studied with some success. this group of organisms (2, 31, 41, 51, 90, 92, 115) pointing out their frequent occurrence in a variety of sources. Of these workers, Weiss and Mercado (115) were the only ones who attempted to name their strains, following a classification drawn up by Prevot (84). It was generally concluded that his was the only serious attempt at classification. This work was published in 1925. Previous attempts to differentiate species had been based on the production of gas and odour, turbidity or precipitating growth in broth, size of cocci, chain formation. metabolism and inconstance of anaerobic requirements. Prevot made a careful detailed study of twenty-seven strains and found the characters listed above to be insufficient for classification. He described fully their morphology, physiology, cultural characteristics in a wide range of media, proteolytic and saccharolytic activity and pathogenicity for laboratory animals. A key to his classification follows:

M. foetidus		Gas in ordinary media. Granular growth. No turbidity Str.anaerobius	2. No gas in ordinary media. Gas in bioprotein media. Turbidity. Str.putridus	oagulated. Str.micros	coagulated. Str.intermedius	Str. evolutus
a) Diameter 0.8 - 1 micron. Short chains in neutral broth. One line of division. Granular growth. No turbidity		b) Diameter 0.8 micron. Long and regular chains in neutral broth No	(2. No) Gas) Tur) a) Diameter 0.3 micron. Milk not coagulated. Gelatine not liquefied.	b) Diameter 0.6 - 0.7 micron. Milk coagulated.	Diameter 0.7 micron. Milk coagulated. Gelatine liquefied
Foetid. not ulated.	Gelatine not liquefied.			Not footid) No gas. Not foetid.
A. Strict anaerobe.)I Lenticular colonies)Gas. in deep agar.)Milk						B. Anaerobe by predilection. Alternate zones of growth in deep agar.

The only other study of note is that of Colebrooke and Hare in 1933 (24), who studied sixty strains noting four different types on the basis of rate of growth, colony formation and changes produced in various media. A preliminary attempt at antigenic analysis showed a great diversity of antigenic structure. Topley and Wilson (102) reviewing the work of Colebrooke and Hare remark that attempts to isolate anaerobic streptococci from the normal throat met with failure, and conclude that these organisms have the genital tract as their principal normal habitat.

In 1933 Prevot reviewed the anaerobic streptococci but included the same species he had described in his previous work. It is largely from these two sources that the descriptions in Bergey's Manual (6) are drawn. Two more species have been added, <u>Str. parvulus</u> and <u>Str. lanceolatus</u>. All but the last have had their source or are reported to have their habitat in the upper respiratory tract.

Str. anaerobius (6, 84) forms chains of cocci about 0.8 micron in diameter. The colonies in Veillon's medium are lenticular and of one to two mm. in diameter; gas is liberated. It grows well in broth; is very peptolytic producing gas in peptone water; does not coagulate nor acidify milk and does not liquefy gelatine. It does not attack cooked protein but will attack fresh animal tissue and fibrin producing much gas with the odour of hydrogen sulphide. Neutral red broth is changed to yellow.

Str. foetidus (6, 84) is characterized by large spheres from 0.8 to 1.0 micron in short chains and sometimes

tetrads or zigzag chains. Growth is slow in semisolid medium, the colonies being at first punctiform, then thick and lenticular, growing 1 to 2 cm. below the surface and forming gas bubbles. Blood agar colonies are surrounded by brownish haemolysis. Growth is good in glucose Martin broth producing foetid inflammable gas, but poorer in plain Martin broth with the production of little gas. Gelatine is not liquefied, Litmus milk is neither acidified nor coagulated; indole is not formed. Neutral red broth is changed to yellow. This species turns fresh organs green then black, with disintegration and liberation of hydrogen sulphide. Cooked protein is not attacked. Acid and gas are formed in glucose, fructose, galactose and sucrose.

Str. putridus (6, 84) occurs in chains of spheres with an average size of 0.8 micron. It forms lenticular colonies but no gas in Veillon's semisolid medium. Colonies on blood agar are surrounded by a blackish brown zone and may themselves become brownish or blackish. The organism grows well in broths, but poorly in peptone water. Gelatine is not liquefied. There is no hydrogen sulphide produced in ordinary media, but its production is very marked in blood broth, blackening the blood. Fresh fibrin is partially digested; neutral red is changed to yellow; lead media is blackened. Glucose, fructose and maltose are fermented to acid.

Str. micros (6, 84) is, morphologically, in long chains or pairs of small spheres from 0.2 to 0.4 microns.

The colonies in Veillon's medium become fairly large (2 - 3)

cm.) growing two or three cm. below the surface and forming processes into the medium. It does not haemolyse blood agar, does not liquefy gelatine, does not form indole, and does not acidify nor coagulate milk in which it grows with difficulty. It does not attack proteins, but produces acid from glucose, fructose, galactose, sucrose and maltose.

Str. parvulus (6). The cocci are small, from 0.3 to 0.4 microns, in short chains, sometimes in pairs. Colonies in deep glucose agar are very tiny, lenticular, whitish becoming black. No gas is formed. There is no liquefaction of gelatine. Indole is not produced, milk is rapidly coagulated, egg white is not attacked, but the organism feebly ferments glucose and lactose.

Str. intermedius forms very long chains in culture, the spheres being from 0.5 to 0.7 microns. Colonies in semisolid agar are regular, lenticular, sometimes bearing complicated processes. There may be slight greening of blood agar. There is poor growth in and no liquefaction of gelatine. Growth is good in broth with acidification of the medium. Milk is strongly acidified and coagulated. Proteins are not attacked; neutral red broth is changed to yellow. Acid is formed in glucose, fructose, galactose, maltose and lactose.

Str. evolutus is a strict anaerobe on primary isolation but becomes facultative with transfers. It occurs in pairs or short or long chains. Spheres average 0.7 micron and are pleomorphic, sometimes appearing as short ovoid rods. Colonies in deep agar are lenticular or rosette in form and

appear about one cm. below the surface of the medium and may form the characteristic alternate zone appearance. Growth is flocculent and heavy in broth and peptone water. Gelatine is liquefied. No indole is formed. Litmus milk becomes acid and curdles, the clot fragmenting. Egg white is not attacked. Much acid is produced in glucose, fructose, galactose, sucrose, lactose, maltose and sometimes arabinose.

Isolation of Streptococcus

McDonald et al (64) isolated their strains of anaerobic streptococci on dextrose blood agar plates, and employed
brain broth for carbohydrate fermentation. Weiss and Mercado
(115) found their strains grew well on hormone rabbit blood
agar, serum agar and Levinthal agar. Blood, ascites and
liver infusion agar were used for isolation by Sears and
Vinton (92) who kept their strains in tubes of chopped beef
heart broth.

PART III DISCUSSION AND CLASSIFICATION OF THE ANAEROBIC BACILLI

The non-sporulating anaerobic bacilli are but little better known today than they were thirty years ago (26).

McCoy and McClung (63) listed some two hundred and sixty-six species of non-spore-forming anaerobic bacilli with eight hundred and thirty-one references. Of these, few have received much attention. Most of the information has been obtained from clinicians who isolated and described new species with descriptions so inadequate that the same organism discovered by another investigator has been given a new name.

No two workers have used the same technique in isolation and

Veillon and Zuber (114) are generally credited for having done the most important early work. They undertook systematic research of several disease processes which yielded several species of anaerobic non-spore-forming bacilli including B. ramosus, B. serpens, B. fragilis, B. perfringens, B. fusiformis and B. fureosus.

In 1919, Castellani and Chalmers (cited in 85) proposed the term Bacteroides to contain the obligatory anaerobic bacilli which were non-spore-forming, not fluorescent, non-pigmented and which grew well on ordinary media. Until this time, the organisms had been classed in the genus Bacillus, which was now reserved for spore-bearing forms. Bacteroides was adopted as a genus, although it contained a variety of different organisms.

forming bacilli was begun in the early 1930's by Eggerth and Gagnon (30) who undertook a study of the anaerobic flora of the intestinal tract. They found that the predominating organisms consisted of Gram negative and Gram positive bacilli. They considered that differentiation on the basis of the Gram stain was a fundamental one, as the Gram positive organisms either formed bifid ends or grew in chains and pairs, or had the aspect and arrangement of the diphtheria group, while the Gram negative strains were more characteristically single organisms, often oval in shape and frequently staining more heavily at the ends or around the periphery. Biochemical tests supported this differentiation. Gram positive strains rarely fermented pentoses, Gram negative ones often did. Gram

negative rods rarely fermented higher alcohols, Gram positive often did. Gram positive strains were more proteolytic and produced a flocculent growth while Gram negative ones produced even turbidity. These workers separated Gram negative strains on the basis of carbohydrate fermentation, finding agglutinations unsatisfactory. Eighteen species were named.

Differentiation of Gram negative non-sporing anaerobic bacilli was attempted by serological means by Weiss and
Rettger (116). The results were quite clear cut with some
exceptions due to cross agglutination. They separated
Bacteroides from the fusiform bacilli on the basis that the
latter were thought to be more rapid in growth, have longer
cells often tapered or possessing granules, and require more
highly specialized media. Weiss and Rettger could not confirm the extensive species differentiation of Eggerth and
Gagnon. They separated their strains into four serological
groups of Bacteroides, supported by morphological characters.

A small series of stool cultures was carried out by Misra (66) which confirmed the results of Eggerth and Gagnon, Weiss and Rettger, with Gram negative strains falling into the same general groupings. Henthorne, Thompson and Beaver (42) isolated strains from pelvic, hepatic, sacral abscesses, etc., naming them on the basis of agglutination aided by morphology and cultural characteristics.

In 1935, Eggerth (29) studied more fully the Gram positive strains of non-spore-forming bacilli from stools.

If the original definition of Bacteroides were to be accepted, these strains would fall into this genus, but he considered

them too different, for the reasons mentioned in his previous paper (30) and thought they might be related to the lacto-bacilli, or possibly constitute a new genus in the order Actinomycetales. One hundred and thirty strains were separated into eleven species on morphological, cultural and fermentative properties. Fermentation tests yielded such diverse results that he carried out single cell isolations to determine the purity of his strains, and obtained the same results.

In 1938, Prevot (75) proposed the suppression of the term Bacteroides for insufficient accuracy and absence of unity among the strains it was supposed to encompass. Under its definition some one hundred and twenty unlike species including Bacillus, Bacterium, Haemophilus, Actinomyces, Corynebacterium, etc. could be classed together, while other organisms with similar characteristics had to be excluded. He completely regrouped the anaerobic non-spore-forming bacilli. His grouping into orders Bacteriales and Actinomycetales was based on general morphology, into four families on the Gram reaction and into seventeen genera on the basis of more specific morphology, capsules, size, involution forms, motility.

Order	Family	Genera
Bacteriales	Ristellaceae)Ristella, Pasteurella, Dialister
	Gram negative)Capsularis, Zuberella
	Bacteriaceae	Eubacterium, Catenabacterium
	Gram positive)Ramibacterium, Cillobacterium
Actinomycetales	Spherophoraceae)Spherophorus, Spherocillus
	Gram negative)Fusiformis, Fusocillus
)Pseudoleptothrix
	Actinomycetaceae	Actinobacterium, Bifidibacterium
	Gram positive)Corynebacterium

Prevot included in his report a description of each species. Unfortunately, he accepted inadequate descriptions of too few strains upon which to base his classification. It is the opinion of Lewis and Rettger (59) that, although Prevot clarified some of the more general aspects of the problem, he has, due to confusing and sometimes arbitrary methods of grouping, brought increased complexity of detail to an already badly confused problem.

These latter workers did not attempt to name species which they isolated from human stools, but tried to show a systematic relationship between them. These strains, as well as others received from Eggerth and Gagnon, and isolated from rat faeces, together with some strains of Lactobacilli fell into two general groups. Group A. comprised Gram positive, non-spore-forming, non-motile, non-capsulating rods; singly, in pairs or short chains; gelatine not liquefied; acetyl-methyl carbonil, indole and hydrogen sulphide not formed; catalase

absent; nitrite not usually produced from nitrates; acid commonly produced from glucose, but not from rhamnose; obligate anaerobes. This group was divided into three sub-types on the basis of morphology, colony characters, reaction to Litmus milk, gelatine, carbohydrates, temperature resistance and minimum, maximum and optimum growth temperatures.

Group B. encompassed Gram negative, non-spore-forming, non-motile, non-capsulating rods; singly, pairs, occasionally short chains; dense turbidity and some sediment in broth; gelatine variable but usually not liquefied; nitrate usually not reduced to nitrite; acid and often gas from glucose, rarely from mannitol; acetyl-methyl carbonil and catalase absent; obligate anaerobes. This group was subtyped as was Group A.

Lewis and Rettger suggested that Group A, types one and two which were straight rods without branching, definitely resistant to high temperature, strictly anaerobic and typically unable to coagulate milk should be regarded as belonging in the genus Lactobacillus. A few strains of Eggerth's fitted into this group. Bifidus types, frequently showing true branching, suggested a relationship to the Actinomycetales.

Group B, type one resembled <u>Bacteroides funduliformis</u>, as described by Henthorne, Thompson and Beauer. Type two comprised a somewhat heterogeneous group of organisms previously described as Bacteroides, but lack of correlation in variable characters made it impossible to arrive at clear-cut subdivisions.

Strains of <u>Bacterium necrophorum</u>, <u>Bacterium pneumo-</u>
sintes and a Fusobacterium were physiologically quite different
from this Group, and growth was poor when attempted on media

satisfactory for the other Gram negative non-spore-forming bacilli. Attempts to show definite relationships between intestinal non-sporulating anaerobes and oral fusiforms were disappointing.

King and Rettger (53) concluded that Gram negative species could be left in a redefined genus of Bacteroides. The Gram positive species were left without classification unless serious consideration could be given the complicated scheme of Prevot (85), or unless they could be included as an anaerobic subdivision of Lactobacillus. These workers studied comparatively the cultural, physiological and serological characteristics of the strictly anaerobic Gram positive bacilli of faecal origin and the oxygen-tolerating Lactobacilli. Similarities were found in some colonial morphology, Gram staining properties, cell morphology, cultural and physiological properties and re-However, there were differences between spiratory enzymes. the two groups in their resistance to potassium cyanide, indole and phenol, and in cell size, serological and skin sensitivity Relation of free oxygen to growth constituted the only criterion of far reaching importance in the separation of the King and Rettger concluded that the anaerobic two groups. bifidus forms, at least, could be included in the genus Lactobacillus.

The anaerobic non-sporulating bacilli are classified by Roy and Kelly as follows in Bergey's Manual, Sixth Edition.

Order Family Tribe Genus

Eubac teriales XI Parvobacteriaceae III Bacteroideae 1. Bacteroides

2. Fusobacterium

TV Hemophileae 4. Dialister

VII Lactobacteriaceae II Lactobacilleae 1. Lactobacillus

The genus Bacteroides has been redefined to exclude Gram positive species, but includes twenty-three Gram negative species. Many of the species described by Prevot in the genera Eubacterium, Catenabacterium, Ramibacterium, Cillobacterium and Bifidibacterium are similar in many ways to the Lactobacilli and although it has been thought that some Gram positive species might be Lactobacilli or related to the Actinomycetes, no place has yet These genera are included in an Appendix been found for them. to Lactobacillus in Bergey's Manual. The validity of Prevot's Gram negative genera Ristella, Pasteurella, Zuberella, Capsularis, Spherophorus and Spherocillus has not been decided upon. are included in the Manual in an Appendix to the genus Bacteroides. genera Fusiformis and Fusocillus appear to be related to the accepted genus of Fusobacterium and some species are included in His genus Dialister is valid. it.

Each genus including non-sporulating rods will be discussed briefly, with regard to species isolated from the upper respiratory tract, methods of isolation and further taxonomic considerations which have appeared recently in the literature.

(a) Bacteroides

Thista and Jonsen (101) after reviewing the literature remark that one sees few of the Bacteroides described by

Eggerth and Gagnon recovered by other workers, and conclude that the number of Bacteroides must be very great or that the methods of classification of species must be of little value. These authors state that so far only a few species have been well defined. These include B. fragilis (found in natural cavities of man and in different pathological conditions) B. funduliformis (otitis, lung gangrene, etc.) and B. serpens (appendicitis, mastoiditis, lung gangrene). In addition there have been reported B. necroticus, B. floccosus, B. mortiferus, B. freundii and B. bullosus, all of which, according to these investigators are different from B. funduliformis so slightly as to make distinction between them ridiculous. Another group of Bacteroides is that of non-pathogens of rather doubtful characterization and found by only one worker. They fall, presumably into different species but the characters thought to be distinct for each species are of very doubtful value.

The buccal cavity is believed to be the natural habitat of organisms of this genus. Lewis and Rettger (59) pointed out that the organisms are associated with upper respiratory tract conditions, appendicitis, colitis, genito-urinary disorders and bacteremia. It has been thought (52) that these bacilli might be the common etiologic agents in tonsillar and peritonsillar abscesses, although occurring as saprophytes in body cavities.

There have been occasional reports of organisms of this type isolated from the buccal-pharyngeal cavity.

Tunnicliff recovered Bacillus gonidiaformans (107) (Spherophorus

Prevot) and <u>Bacillus rhinitis</u> (106) (Zuberella Prevot) from the throat. Prevot isolated an organism he named <u>Ristella</u> <u>distillationis</u> from a case of bronchitis.

One species of Bacteroides, B. melaninogenicum has been well established as a common inhabitant of the oral cavity. It was originally described by Oliver and Wherry (79) and has been fully studied by Shevky, Kohl and Marshall (94). In direct smear the organism appears much like a diplococcus; in fluid media it is pleomorphic, forming clumps of bacilli. It loses the stain by Gram's method and is not acid fast. Primary cultures are visible after one to two weeks incubation anaerobically at 37°C. on human blood agar; pure cultures appearing as a confluent black dry layer. As growth increases the haemoglobin in blood disappears. Pigment is extracellular melanin and depends on haemoglobin for its pro-A fairly luxuriant white, moist growth is obtained duction. on Loeffler's blood serum. The species is non-motile; does not liquefy gelatine; does not reduce nitrates; produces indole and acidifies and peptonizes milk. Acid is formed in dextrose, levulose, lactose, saccharose, maltose and mannite phosphate broth. Not all media allow fermentation.

Isolation of Bacteroides

For isolation of Bacteroides, Eggerth and Gagnon (30) employed a basic medium of agar, peptone, di-sodium phosphate with 5 per cent sterile blood and 0.15 per cent sterile glucose added. Stock cultures were kept in brain medium. Weiss and Rettger (116) found beef infusion agar with 7 per cent defibrinated cow's blood and 0.15 per cent glucose satisfactory.

Egg meat was employed as a stock medium. Blood plates were also used for isolation by Henthorne and co-workers (42). Lewis and Rettger (59) found the use of three media resulted in satisfactory isolations. These consisted of the beef infusion agar, a glucose-cysteine medium and a beef infusion agar containing potato extract, serum, salt, peptone, savita and glucose. Growth facilitating factors were studied by these last two workers in cooperation with Bedell (58). Optimum growth temperature was found to be 35 - 40°C., and the optimum pH 6.3 - 7.0. The addition of carbon dioxide to the anaerobic system was found to stimulate growth, and a medium of tryptone, beef extract, glucose, cysteine hydrochloride, disodium phosphate and water was found most satisfactory.

(b) <u>Fusobacterium</u>

Tunnicliff (104) cites references which stated that there is but a single species of fusiform bacillus, but this has met with disagreement by many workers. Krumwiede and Pratt (54) in 1913 divided fifteen strains into two groups on the basis of sucrose fermentation. Knorr is said (99) to have recognized three morphological types, Pratt (83) two, supported by sucrose fermentation. Three morphological groups were also recognized by Smith (98). Varney (113) attempted a serological classification, distinguishing between four morphological and serological groups having a characteristic colonial Fifty-three strains were separated into four appearance. morphological, cultural and biochemical types by Slanetz and Rettger (95) who found Varney's serological methods unsatis-The first study in which both biochemical and factory.

serological methods were employed was attempted by Spaulding and Rettger in 1937 (99). Two groups were distinguishable by fermentation reactions. Each group divided into two types which were partly confirmed by agglutination tests. The four types included those of Slanetz and Rettger. During the same year, Hine and Berry (43) studied one hundred and four strains from the mouth finding that three morphologically distinct groups could be isolated from almost every adult mouth. They noted, however, that morphology alone was not sufficient for identification because of the pleomorphism of the genus. They described three species, each including some of the types described by Varney, Smith, Slanetz and Rettger, and Knorr. In Prevot's scheme (85) the fusiform bacilli were included in two genera, Fusiformis and Fusocillus and numbered five species.

Bee (13) realized that it was impossible to find agreement among the results of previous workers. He felt that factors such as bacterial pleomorphism, difficulty in cultivation and variable adaptability to new growth conditions had contributed largely towards an unsatisfactory classification. The group "fusiform rods" had been poorly defined and Bee thought there had been particular confusion with the Lepto-With reference to serological classifications, he trichia. felt that the workers who had attempted it (Varney, Slanetz and Rettger) had probably confused Leptotrichia and Fusobacterium and had established serologically distinct groups on this Even in cases undoubtedly involving Fusobacterium, hasis. Bee had the impression that different forms of the same organism have been described as distinct species. He stated that

classification on the basis of growth characteristics was irrational, for although strains showed marked variations with regard to the medium in the first generations, these disappeared rapidly, and were found in strains which were otherwise identical. Morphology was thought to be of little use in classifying the fusiform group as each strain might show extreme variation. Motility, also was thought to be unsuitable as a basis for classification because although motile strains might occur the majority were not motile or had such sluggish motility that it could not be confirmed by ordinary Biochemical reactions for Boe's thirty strains were clear cut and unmistakable, and uniform for all strains. This afforded an easy and reliable distinction from the Leptotrichia but could not serve as a basis for type classification. Biochemical reactions which were significant in the identification of the Fusobacterium were as follows. There was constant and active production of indole, production of hydrogen sulphide, weak fermentation of carbohydrates to a terminal pH over 6.0, no liquefaction of gelatine nor formation of gas. Bee regarded the indole negative and highly saccharolytic strains of Spaulding and Rettger as Leptotrichia. Serological reactions which he himself obtained gave a clear cut distinction between Fusobacterium and Leptotrichia, but type classification of Fusobacterium was not satisfactory, as this group of bacteria was found to have a complex antigen structure and a pronounced strain specificity. For the present, this author concluded that it was justifiable to recognize only one species, Fusobacterium planti-vincenti (Knorr).

Breed, however, in the Sixth Edition of Bergey's Manual (6) recognizes four species in the genus Fusobacterium. Three have their habitat in the upper respiratory tract. Previous classifications of these (43) are given.

F. nucleatum - Hine and Berry F. nucleatus Knorr F. nucleatus Varney Group III Smith ... Group III Slanetz and Rettger Group I F. polymorphum - Hine and Berry F. polymorphus Knorr F. polymorphus Varney Group I and III Smith Group II Slanetz and Rettger Group II F. plant-vincenti - Hine and Berry F. dentium Knorr F. plant-vincenti Varney Group IV Smith Group I Slanetz and Rettger Group III and IV

Fusiform bacilli are common in the mouth. Hotchkiss (47) found them in one hundred and twenty-one of one hundred and sixty mouths, and their presence there and in the throat has been confirmed by many other workers (43, 45, 83, 91, 98, 99, 111). Tunnicliff (104) noted that most bacteriologists agree that fusiform organisms isolated resemble those found in Vincent's infections. A few are normally in the mouth, and appear in large numbers in ulcero-membranous conditions.

A brief description of the three respiratory tract species follows:

F. planti-vincenti (6, 43). The rods are 0.5 to 1 by 8 to 16 microns occurring in pairs with blunt ends together and outer ends pointed, sometimes in short curved pairs, or long spirillum-like threads. Granules are present. The organism is Gram negative; non-motile. Colonies in deep serum agar are spherical, up to 0.5 mm. in diameter and are yellowish brown. Growth on serum agar is matted, turbid in serum broth, precipitated in liver broth. No indole, hydrogen sulphide, odour nor gas is produced. Glucose, fructose, sucrose, maltose and sometimes lactose are fermented to acid.

F. nucleatum (6, 43) is, morphologically a Gram negative, spindle shaped bacillus of one by four microns, occurring singly. One or two granules are present. The species is non-motile. Deep colonies in serum agar are lens-shaped, with off-shoots. There is growth in liver broth containing serum, but none in plain liver broth. A disagreeable odour but no gas is produced. No hydrogen sulphide is formed. Glucose and sometimes fructose, sucrose and lactose are fermented to acid.

F. polymorphum (6, 43) is in the form of rods of 0.2 to 0.5 by 8 to 16 microns in pairs with pointed ends adjoining and often in threads. It is Gram negative and non-motile. In alkaline serum agar the colonies are lens-shaped with off-shoots. The organism forms a tenacious sediment in liquid media. No gas, no odour, no hydrogen sulphide is formed. Glucose, fructose and sucrose are usually fermented.

Isolation of Fusobacterium

The isolation of fusiform bacilli was carried out by Varney (113) on blood agar, the base containing proteose peptone, beef extract and salt. He kept stock cultures in brain medium under vaseline seal. Slanetz and Rettger (95) found an anaerobic technique with the addition of carbon dioxide stimulated growth. The most satisfactory medium for isolation and holding of strains incorporated potato extract and gentian violet in a dilution of 1:20,000, in addition to meat extract, proteose peptone and sugar. Stock medium included gelatin. This medium was found more satisfactory than one containing blood or serum. The same medium, with the addition of cysteine hydrochloride and glucose was employed by Spaulding and Rettger (99) and yielded pure cultures of fusiform bacilli. The need for absolutely fresh medium was stressed. A study of growth requirements by these same workers (100) showed that ordinary laboratory media would not support growth. The addition of cysteine hydrochloride was invaluable for its reducing property. Hine and Berry found no single medium was ideal for primary isolation, some of the organisms being most easily recovered on 5 per cent rabbit blood agar containing gentian violet 1:5000, others on the potato extract medium of Slanetz and Rettger.

1) Inter-relationships

As previously mentioned, Bee (13) maintained the view that classification studies of the Fusobacterium had been obscured by the inclusion of strains of Leptotrichia. He claimed that the latter is distinct serologically, highly

saccharolytic, and a non-producer of indole. He thought the two genera should be placed near each other in classification as they seemed closely related. Prevot (85) placed them as co-ordinate genera in his new family Spherophoraceae (Fusiformis, Fusocillus, Pseudoleptothrix) including in this family the species Spherophorus necrophorus (Actinomyces necrophorus Bergey) and Spherophorus funduliformis (Bacteroides funduliformis). The distinguishing characteristic of Spherophoraceae was the formation of spheroid bodies which Boe claimed were probably rarely if ever produced by Fusobacterium, and never by He concluded that the logical consequence was Leptotrichia. to place Fusobacterium and Leptotrichia as two co-ordinate genera in the Family Bacteriaceae. He thought that further investigation might lead to the placing of the two genera in a distinct tribe on the basis of one common characteristic - the tendency to form threads.

ject of much confusion. The editors of Bergey's Manual no longer recognize it as valid, as few of the species assigned to it have been well enough described to be accepted. Furthermore, the type species <u>L. buccalis</u>, which has been most frequently described has often been described as Gram positive. The genus is included in an appendix to Lactobacillus in the Manual. A description of <u>L. buccalis</u> follows.

L. buccalis (89). Gram positive unbranched filaments, non-motile, non-spore-forming; tending to grow as coarse and often granular threads or long rods which may be rounded and sometimes clubbed at one end, tapered at the other. It becomes

Gram negative bearing Gram positive granules with age. An atmosphere of carbon dioxide proves most suitable for growth. Growth can be obtained on brain medium containing agar, colonies having a characteristic thatched appearance and pearly luster. On blood agar the colonies are smaller, more irregular and may produce slight greening. Milk is acidified, gelatine is not liquefied, no indole nor hydrogen sulphide is produced. Several carbohydrates are fermented.

Throughout this review there have been several references to the relation between certain Gram positive rods and the Actinomycetes. As the latter are characterized by the formation of a mycelium they are classed by some authorities with the fungi, although in Bergey's Manual they are placed in a family in the order Actinomycetales. (89) reported that the differences between A. israeli and L. bifidus appeared quantitative rather than qualitative, and thought that the Actinomyces might bear a bond of relationship to the bacteria proper through Lactobacilli of this type. natural habitat of the one parasitic species Rosebury recognized, A. israeli is the mouth and throat and there is little wonder that there has been confusion in attempts to classify Although A. israeli produced branching mycelium it strains. was found to vary markedly under different conditions. mouth and throat there were several forms, no clubs and rare In culture there appeared anything from a compact branching. mass of branching mycelium of Gram positive filaments to short. regular evenly staining or granular non-branching rods. Differences were associated with rough and smooth forms.

Rosebury found isolation and maintenance difficult, the organism requiring alternate transfers to different media. Carbon dioxide in 5 per cent in an anaerobic atmosphere proves most satisfactory for growth, strains showing a varying tolerance for oxygen, but anyone dying out if maintained through several aerobic transfers. Colony appearance varied with colony dissociation. Acid but no gas is obtained from a wide range of carbohydrates.

Other inter-relationships have been reported by Lahelle (55) between Fusobacterium and the Necrobacterium isolated from pathological conditions in animals. reported as anaerobic, Gram negative, non-spore-bearing, often thread formed microbes with granules. Both produced rough, smooth and intermediate forms. The most pronounced cultural difference was the strong haemolysis produced by Necrobacterium. while Fusobacterium was not haemolytic. Biochemical differences appeared small, and they were found to possess common Labelle, however, found the two groups sufficiently different to keep in separate genera. Thjøtta (101) could find no difference, other than source, between Bacteroides funduliformis and Necrobacterium necrophorus. Necrobacterium necrophorus has been classified as Bacteroides (Bergey, 1939) as Spherophorus (85) as Fusiformis (102) and as synonymous with Actinomyces necrophorus by Thiøtta.

The relation between Bacteroides, Leptotrichia and Actinomyces has been supported by other workers (42).

Further work will probably lead to the discovery of a number of serological relationships between many non-sporing rods with a tendency to grow in filaments.

That filamentous forms are common in the mouth has been supported by Bibby and Berry (10) who isolated seven groups from gingivae in adults and children. Four of these groups seem related to Leptotrichia, but others have properties pertaining to more than one genus and their classification cannot be determined.

2) Fusiforms - Spirochaetes

There have been many conflicting views in the literature regarding the relationship of fusiform bacilli and spiro-It had been thought that they were two forms of the chaetes. same organism, that they bear a genetic relationship, and that a spirochaete could develop into a fusiform. Tunnicliff carried out some rather extensive research on the subject (105, 108, 109) finding that spirillar forms appeared in her fusiform cultures in a few days. It was shown that by dissociation a great variety in size and shape occurred; smooth colonies being made up of bacillary forms, while rough dissociants contained filaments, pleomorphic forms and spirilla-like organisms. explanation of dissociation was supported by other workers (98, Slanetz and Rettger (95) found that although threadlike filaments or curved or wavy forms appeared, spirochaetal forms were seen in only one of their fusiform types, seeming to develop from deep staining bodies in the cells. Spaulding and Rettger (100) failed to produce more than an occasional spirillary element. It was finally concluded (44) that there was no evidence of a genetic relationship as spiral elements which did form did not greatly resemble oral spirochaetes, being thicker and showing rigid coils. Comparable size and staining

reactions made it apparent that the organisms were all fusiform bacilli.

(c) Dialister

The previously quoted transmission experiments of Olitsky and Gates in 1920 (70) yielded a non-spore-bearing anaerobic bacillus of a type distinguishable by its characteristic small size. A series of experiments (22, 33, 34, 46, 65, 69, 71, 72, 73a,b,c,d, 74a,b, 75, 76, 77, 78) yielded several types of Gram negative filter-passing organisms, but only one of these has been named with certainty. It was originally named <u>Bacterium pneumosintes</u> but has since been incorporated into the genus Dialister.

Dialister pneumosintes was isolated by the inoculation of nasopharyngeal washings, filtered through Berkefeld V. and N. filters into a special medium of Noguchi consisting of ascitic fluid containing a piece of rabbit kidney under vaseline seal. Material from the bottom of the tube showed minute bodies from 0.15 to 0.3 micron in length, bacilloid rather than coccoid, often singly, sometimes diploform, rarely in short chains. Pleomorphism is not marked. Reaction to the Gram stain is negative. The organism does not attack carbohydrates and alcohols.

A similar organism, anaerobic to microaerophilic has also been isolated from the respiratory tract in influenza (6). Dialister granuliformans passes through a Chamberland L2 filter and is a small non-motile Gram negative rod. It does not affect Litmus, and does not form indole. Acid is formed from glucose, sucrose and mannitol.

(d) Lactobacillus

Classification of this genus is still a matter of controversy. There is no need to repeat again the interrelationships which have been mentioned throughout this review, linking it with other groups of organisms. present it is included in the family Lactobacteriaceae (6). Rosebury (88) groups all the aerobic oral and vaginal forms under the species name Lactobacillus acidophilus, while all the anaerobic strains, or those requiring anaerobiosis, at least for isolation are included under the term L. bifidus. There are probably two L. bifidus. \overline{I} , the classic one described by Tissier which is anaerobic only on isolation, and TI which has been grouped with the Bacteroides (29, 53) and which is permanently anaerobic. L. bifidus is essentially an intestinal form, but has been reported in the mouth (48). Oral forms have not been extensively studied (25, 40, 93). However, Rosebury feels that the distinction between L. bifidus T and L. acidophilus seems to rest only on a difference in oxygen tolerance.

He notes that the Lactobacilli closely parallel the streptococci in morphology, metabolism and habitat. The Lactobacilli tend to become shortened approaching the streptococci form and the streptococci tend to become elongated, approaching the bacillary form.

L. bifidus (6) is morphologically, a small, slender bacillus, averaging 4 microns in length, occurring singly or in pairs and short chains, very variable in appearance, branching and clubbing developing in some cultures. The

organism is non-motile. It forms colonies up to three mm. in diameter with a slightly irregular edge in deep sugar agar. Inactive lactic acid and volatile acid production in sugars is variable.

Isolation of Lactobacillus

The addition of growth stimulating substances and a utilizable sugar (88), whey or glucose (102) or tomato peptone agar (25) have been suggested as an aid to isolation. The character of Lactobacilli of surviving high concentrations of acid has been used for their isolation, as their production of active or inactive acid has been used in an attempt at classification (102).

(e) Spore-forming Bacilli

The Clostridia have been reported to be transient invaders of the mouth. Hall, reviewing the literature noted the findings of butyric acid in saliva suggestive of B. butyricus and the report of rather doubtful validity of the isolation of B. putrifecus. He isolated B. fermentans from pus in a tooth (36) and in a study of fifty-five salivas found six yielded sporulating anaerobes including Cl. welchii, Cl. bi-fermentans, Cl. tetanomorphus, and Cl. tetani (37, 38). He concluded that these organisms were transient saprophytes.

PART IV DISCUSSION OF THE SPIROCHAETAE, SPIRILLUM, VIBRIO

The literature on the spirochaetes is abundant, but limited almost entirely to morphological studies. There have been a few surveys carried out on their occurrence in the buccal-pharyngeal cavity. Hotchkiss (47) found spirochaetes

in ninety-seven of one hundred and sixty-one mouths of healthy young adults. Brooke (18) reviewing the literature in 1938 quoted estimates that the Leptospira might reach 40 per cent incidence and that <u>Treponema vincenti</u> could be found in small numbers in the normal mouth. He reported that it was generally agreed that spirochaetes occurred in 30 per cent of normal mouths, but no identity was given them. Spirochaetes have been found in the healthy adult edentulous mouth (91) and in tonsils (82).

David T. Smith in his text "Oral Spirochaetes and Related Organisms in Fuso-spirochaetal disease" (98) described spirochaetes as being of three types, although they had previously been thought to be of one species. The three types consisted of the free-living, saprophytic and pathogenic forms, the latter being limited to the Treponema and Leptospira genera. Most of the oral forms were thought to belong to the Treponema genus, the tissue residing ones being anaerobic. Smith recognized five common oral species.

The editors of Bergey's Manual now recognize four oral species: Borrelia buccale, Borrelia vincenti, Treponema microdentium and Treponema mucosum. It is possible that other species mentioned by Smith, or in other reports throughout the literature may be identical with these, or deserve recognition but they have been too inadequately described or too unlike known species to be placed in genera.

Differentiation of the above four species is largely based on morphological characters. Cultivation has been accomplished in some cases (6). Smith claimed successful

isolation of a few of his species by a technique of serial dilutions in a stab in the centre of a deep column of agar, the spirochaetes wriggling through the meshes of the agar, forming a pure culture projecting from the core of mixed growth. Fresh serum, ascitic or pleural fluid was necessary Almost all studies, however, have been morphological. Smith stated that dark field examination was the only satisfactory method of identifying them on morphological grounds, as fixing and staining destroyed their characteristic appearance. Brooke, (18) however, claimed that it was possible, with the use of a modified Gram stain to identify seven species.

The genera Spirillum and Vibrio consist mainly of aerobic species. Many of the oral ones have not been described, or described inadequately with no mention of their anaerobic properties. Smith (98) reported the isolation of two anaerobic species of Spirillum, and there have been several species named in reports throughout the literature (6).

The subject does not merit a more detailed discussion in this study, which has been devoted primarily to a cultural examination of bacterial species which do not require special techniques.

PART V PATHOGENESIS

The significance of the normal bacterial inhabitants of the buccal-pharyngeal cavity in the production of disease, has more often been along the lines of conjecture rather than of experimental proof. And yet, that they play a part is probable, for the bacteriology of bucco-dentary infections is almost entirely anaerobic (56) and a glance at the literature

shows how very frequently anaerobes have been isolated from a number of disease processes, many of which could have had their origin in the respiratory tract (59).

Plant and Vincent first incriminated an association of fusiform bacilli and spirochaetal forms in necrotic lesions of the oropharynx. The characteristic picture of Vincent's disease is recognized to be that of fusiform bacilli and wavy spirilla in smears from lesions (104) and while fusiforms are occasionally seen in pyorrheae tissue (3) they are never present in numbers equal to those in ulceromembranous conditions (111). The organisms, however, have been found, in general to possess no greater pathogenicity than those living harmlessly in the normal mouth. Experiments with combined pure cultures offered no conclusive evidence that these two kinds of bacteria were accomplices in producing lesions. It was thought that anaerobic streptococci and vibrios might be involved. None of these organisms alone, or in any incomplete combination proved effective, but combined pure cultures of all four formed necrotic foul-smelling lesions typical of fuso-spirochaetal disease (52, 97). It had been suggested that lowered tissue resistance was the predisposing factor allowing normal saprophytes to become pathogenic. Kelly (52) however showed that tissue injury was not, in itself, sufficient to increase the pathogenicity of the organisms.

The combination of fusiforms and spirochaetes also has been reported to be the cause of ulceromembranous gingivitis, pulmonary abscess, gangrene, noma, pharyngomycosis, pyorrhea alveolaris, alveolar abscess, bronchitis, bronchiec-

tasis, bronchial spirochaetosis, infections from teeth wounds, osteomyelitis of the jaw and sublingual gland infection (5, 12, 27, 82).

The anaerobic, non-spore-forming bacilli may be primarily responsible for the lesions from which they are isolated, but the etiological role of others is probably of a secondary nature (100, 102). Due to the inadequate descriptions of strains isolated from these lesions, it is impossible to designate many of them to definite species or genera, so the group must be regarded as a whole. From available statistics it is seen that these organisms most frequently attack mucous membranes, but under certain circumstances may invade any organ of the body causing necrosis of tissues.

Anaerobic non-sporing bacilli have been recovered from pleural empyema, meningitis, bronchiectasis, gangrene, brain and lung abscess, bronchitis, otitis, pleurisy, surgical wounds and other pathological processes (17, 19, 21, 28, 50, 79, 114, 117).

Septicemias from which non-spore-bearing bacilli have been isolated, often occurring after pharyngeal infections, have been frequently reported (52). Sites from which these organisms are most apt to invade the blood stream are thought to be the tonsil and peritonsillar regions, middle ear, mastoid, teeth and gums (87). Infection occurs after damage to the mucous membrane and probably other conditions, as most of these species appear to have a low degree of invasiveness (26).

Lemierre (57) has mentioned that a thrombophlebitis is a common finding at the site of the initial invasion, and possibly the stasis of blood brought about as a result, establishes con-

ditions which are suitable for the multiplication of these anaerobes.

Most of the anaerobic bacilli appear to be nonpathogenic for laboratory animals (108). Some of Eggerth's
strains (29) produced subcutaneous abscesses in rabbits.

Bacteroides of Reid, Snyder et al (87) from septic infections
resulted in death to guinea pigs.

The micrococci have been recovered from several sources, namely the uterus, skin and tonsils (32), focal infections and appendicitis (86), and the Veillonella from lung lesions, appendicitis and focal infections (86) in addition to the normal respiratory tract. The significance of their presence in such infections has not been determined.

natural body cavities and can become pathogenic when the opportunity offers (84). They are common in many suppurative and gangrenous conditions (92) any of which could have had its source in the upper respiratory tract. The invasive role of anaerobic cocci is apparently increased when they are in symbiosis with other organisms. Barnes and Bibby (2) cite a reference in which it was concluded that the cocci were the aggresive agents in gangrenous pneumonia, which invaded the blood stream while the concomitant fusiform bacilli and spirochaetes remained localized. Anaerobic streptococci have been isolated alone or with other bacteria from dental root granuloma, gangrenous pneumonia, Vincent's infection, teeth wounds, bartholinitis, Ludwig's angina, lung lesions, empyema, pleurisy, pulmonary abscess, otitis, gas gangrene in war wounds, intestinal

tract, meninges and puerperal infections (2, 6, 31, 52, 64, 80, 84, 102). Of these, the last is perhaps of greatest importance (24).

Animal experiments on the anaerobic streptococci have not been numerous. Few workers have shown that they are pathogenic in pure culture. Prevot's strains (84) were rarely pathogenic, some producing slight abscesses in laboratory animals. Henthorne and McDonald (41) pointed out that the streptococci lack pathogenicity when pure, but in symbiosis with other organisms may cause meningitis and ophthalmitis in rabbits. The two hundred and seventy-seven puerperal strains of Colebrooke and Hare (24) produced local lesions but no death in animals.

The bacterial flora of the abscess of the lung has undergone considerable investigation. Bucker (20) concluded that the organisms in pulmonary abscess were for the most part those normally in the mouth and respiratory tract, and believed that entrance was through the air passages. The significance of the flora has not been clearly understood. Marshall and Brunn (62) recovered spirochaetes, aerobic and anaerobic streptococci and fusiforms. Cohen's study (23) of sixteen lung abscesses showed that anaerobic streptococci were in all, Bacteroides melaninogenicum in fourteen, and fusiforms, anaerobic bacilli, V. parvula, and vibrios were common. Varney (112) found that, in untreated chronic lung sbscess, the most common organisms were streptococci, fusiforms, B. melaninogeni-In treated abscesses, fusiforms, spirocum and spirochaetes. chaetes and B. melaninogenicum decreased. The flora showed a

remarkable similarity to that from infected tonsils, cervical abscesses, diseased teeth and mucous membranes.

There have been many theories regarding the cause of dental caries. Many organisms have been isolated but the bacteriological studies are not yet conclusive (48). Almost every acid-producing organism has been accused. Bibby and Hine (11) could see no evidence from smears of carious cavities of any morphological group of organisms being of primary importance. The weight of evidence seems to point to the Lacto-Snyder (96) found that children with no Lactobacilli in their saliva were free from caries, while those with significant numbers had caries. Rosebury (88) also implicates the Lactobacilli. The organisms have been observed to increase markedly during the onset of caries of the enamel. He found that they are capable of decalcifying enamel and Unfortunately, as most of the oral Lactobacilli are dentin. aerobic, one can associate with less assurance the anaerobic Aside from association with dental caries, the strains. Lactobacilli are not known to be pathogenic.

SECTION B.

INVESTIGATIVE WORK

PART I METHODS AND MEDIA USED IN ISOLATION OF STRAINS

Material to be studied was obtained from thirty patients in the Children's Memorial Hospital. Their ages ranged from eight days to thirteen years, and they were hospitalized for a variety of complaints including diarrhoea, tuberculosis, rheumatic fever, bronchiectasis, bronchial asthma, tonsillitis, common cold. None was receiving drug or antibiotic therapy and had not received any for at least two weeks. The subjects will be designated by letters of the alphabet throughout this discussion.

Specimens for smear and culture were obtained by the following methods. Material from the nose, throat and mouth was obtained by the use of absorbent cotton swabs and taken from the back of the throat and from around the gums. teeth and saliva. Nasopharyngeal suction was carried out on a number of cases to obtain mucous. The apparatus (1) used consisted of a twenty ml. syringe to which was fitted a two inch length of rubber tubing with a hole cut in it. A seven inch French number nine or number ten catheter was attached to the rubber tubing by a two inch glass tube. The apparatus was prepared for use by washing in running water and sterilized by autoclaving. When in use, the barrel of the syringe was held in the left hand, with the left thumb closing the hole in the rubber tube. The right hand inserted the catheter to the nasopharynx. Suction was started with the thumb over the hole, the piston released with the thumb off the hole.

pushing and withdrawing the plunger several times, mucous was aspirated up into the catheter and was visible through the glass tube. Nasopharyngeal suction, nose and throat swabs were obtained from the first patients, F, G, H, I and J. Patients K. to U. inclusive had a mouth swab taken in addition.

At this point, it was decided to abandon the nasopharyngeal suction method of obtaining culture material, as
it seemed to have no advantage over swab taken specimens, particularly as mucous was often very scanty in the nasopharynx.
Remaining specimens were obtained by swabs of the nose, throat
and mouth. A West swab was used once or twice but found impractical in small children.

Immediately upon returning to the laboratory cultures were made.

Material from patients F. to U. inclusive was streaked to a 4 per cent human blood agar plate, which was spread by a bent wire spreader to two others. The swab or mucous was then dropped into anaerobic thioglycollate medium.

Potato extract medium, used by Spaulding and Rettger (99, 100) for the isolation of fusiforms was employed in addition to the above for cultures V, W, X, and Y.

The remaining specimens were streaked also onto Difco tomato medium, as an aid to the isolation of Lacto-bacilli (25).

These media, the formulae of which appear in Appendix II, were considered adequate to supply the growth requirements of most of the known anaerobic bacterial species which had been

encountered in the upper respiratory tract in previously reported work, with the exception of the Spirochaetae, and the genus Dialister, for which special techniques would have to be employed. It was not considered possible to attempt these for this study.

Smears were done on materials from patients V. to MM. inclusive; duplicate swabs being obtained for this purpose. The stain used was the modified Gram method proposed by Brooke (18) which reportedly showed spirochaetal morphology and retained the differential value of the Gram stain. This staining method consists of mixing 5 per cent sodium bicarbonate and 1 per cent gentian violet in the proportion 4: 15 and allowing the fresh mixture to act seven minutes. The slide is washed in running tap water, Gram's iodine allowed to act for three minutes, the slide washed, decolourized, and counterstained with 1 per cent carbol fuchsin for forty seconds. Gram positive organisms are stained a deep reddish purple, Gram negative ones pink.

and the thioglycollate meat medium under ordinary atmospheric conditions at 37°C. An undertaking of this kind necessitated the choice of anaerobic apparatus which would accommodate large numbers of plates and slopes, and be rapid, efficient and safe. Moreover, the form of colonies on the medium surface could be studied to better advantage, than anaerobic methods employing deep medium cultures. Since the original McIntosh and Fildes anaerobic jar was described in 1916 many modifications have been suggested. Two types were used in

this study. The first was a Brewer modification of the Baltimore Biological Laboratory, the other a modification by Professor E. G. D. Murray of McGill University. Anaerobic conditions were checked by placing a tube of methylene blue dextrose broth in the jar. Carbon dioxide was added to the jars for all cultures from V, as recommended for isolation of Fusobacterium (95), Bacteroides (58) and Lactobacillus (25).

Plates were examined after two, five and seven days' incubation. Each type of colony visible by hand lens was picked with a straight platinum wire to slants of 4 per cent human blood agar. The thioglycollate meat medium was subcultured to a blood agar plate in two days, colonies being picked after three, five and seven days. The inoculated blood slants were incubated anaerobically, and when growth occurred were subcultured to blood agar plates aerobically. The strains subcultured from plates of patients F. through Q. which showed no aerobic growth were then picked off to fresh thioglycollate meat medium, incubated until growth appeared and kept at room temperature for future study. Cultures from R. through MM. showing no aerobic growth were subcultured to thioglycollate meat medium as before, smeared, stained by Gram's method, and rechecked aerobically to rule out error. Cultures F. through Q. were subsequently replated and checked by smear and aerobic culture. A few strains were inoculated also onto Loeffler's blood serum or blood agar to insure All slants were kept under anaerobic conditions, growth. while aerobic growth checks were in progress. If no growth occurred on the inoculated slants, they were kept a minimum

of one week before discarding. An average of over one hundred colonies was picked per subject.

All cultures in thioglycollate meat medium were subcultured to fresh media every two weeks. Finally, all cultures were streaked to blood agar plates and incubated anaerobically. Well isolated colonies were picked, smeared and replated to determine purity. In some cases, three or four replatings were necessary to ensure purity, because of the pleomorphism of some strains and a tendency for some bacteria to grow in symbiosis. Replating from three to six times was advocated by Eggerth and Gagnon (30) for Bacteroides strains.

When purity was established each strain was streaked to a blood agar plate and colonial morphology was examined after three days, and checked afterwards each time the jar was opened, after incubation at 37°C. A colony was picked to a blood agar slope, and bacterial morphology determined by Gram's stain after one and five days incubation. Subcultures were made to thioglycollate meat medium for identification.

PART II METHODS AND MEDIA USED TO IDENTIFY STRAINS

On the basis of general morphology and reaction to the Gram stain, the strains isolated were separated into six groups: Gram positive cocci in clusters, Gram negative cocci, Gram positive cocci tending to form chains, Gram negative bacilli, Gram positive bacilli, Gram positive spore-forming bacilli.

The following outline presents the detailed results obtained by smear and colonial morphology examinations of strains falling into each of the above groups. The source of each is given together with the medium used in isolation, history of the subject from which a strain was recovered, methods and media employed for biochemical and other tests, and an attempt to identify strains by comparison with previously described species. For the source, nose, throat and nasopharynx are grouped together as respiratory tract. Formulae for special media appear in Appendix II.

A few of the strains did not survive transfer, and complete examinations could not be made. The results that were obtained as far as was possible are given.

All strains were anaerobic on isolation, and all remained so except when noted otherwise.

(a) Gram Positive Cocci in Clusters

Examinations appropriate to the anaerobic Micrococci were carried out following the criteria presented in Bergey's Manual (6) and Prevot's article (86). A table of results appears in Appendix $\overline{\underline{\mathbf{I}}}$.

- Gelatine: 5 ml. Difco gelatin incubated anaerobically, read in seven days.
- Deep agar: Tubes of peptone agar to a height of 10 cm., melted, cooled to 45°C., inoculated and incubated aerobically.

 Examined daily.
- Deep serum agar: Tubes of peptone agar containing 10 per cent human serum, treated as above.
- Deep dextrose agar: Tubes of peptone agar containing 1 per cent dextrose, treated as above.

- Deep neutral red serum agar: Tubes of 10 per cent rabbit serum peptone agar containing 0.1 per cent of a neutral red saturated solution, treated as above.
- Dextrose blood broth: 5 ml. peptone broth containing 1 per cent dextrose and 5 per cent human blood, with lead acetate paper protruding through the plug, incubated anaerobically. Examined in two and seven days.
- Dextrose serum broth: 5 ml. peptone broth containing 1 per cent dextrose and 5 per cent human serum, incubated anaerobically. Examined in two and seven days.
- Nitrate reduction: 5 ml. nitrate broth, incubated anaerobically and tested in five days by the sulphanilic acid di-methyl alpha naphthylamine method.
- Peptone serum water: 5 ml. peptone water containing 5 per cent rabbit serum, incubated anaerobically. Examined in two and seven days.
- Milk: 5 ml. Difco Litmus milk incubated anaerobically and examined after two and seven days.
- Coagulated protein: Loeffler's blood serum slants, coagulated egg white and coagulated human serum, incubated anaerobically and examined in two and seven days.
- Neutral red broth: 5 ml. peptone broth, containing 0.1 per cent of a saturated solution of neutral red, incubated anaerobically and examined in two and seven days.
- Fermentations: 5 ml. Hiss serum water containing 0.5 per cent of lactose, sucrose, dextrose, maltose, mannitol and starch, incubated anaerobically and examined in two and seven days.

P. 43 Source: Mouth.

Medium: Blood agar plate.

Subject: Male; one month; diarrhoea.

Smear: Small Gram positive cocci, singly, pairs and clusters.

Colony: Pin point, round, raised, colourless, transparent, soft.

Did not survive transfer.

P. 60 Source: Mouth.

Medium: Blood agar plate.

Subject: as P. 43.

Smear: Very small Gram positive cocci, mostly in clusters and pairs. A few of the elements were slightly pleomorphic, tending to be elongated.

Colony: 1-2 mm. in diameter, round, raised, greyishwhite, opaque, soft, slightly alpha haemolytic.

Resembles <u>Micrococcus anaerobius</u> in many respects but colonies in deep agar were small, rather than thick and yellow. Indole production was slight, and milk was slightly acidified rather than negative. With the exception of maltose, the carbohydrates in peptone water were not fermented, as <u>M. anaerobius</u>, but in Hiss serum water, acid was produced from them all. This strain is not named.

V. 3 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male, seven weeks, meningocoele

Smear: Small cocci, singly, pairs of slightly elon-

gated units, clusters.

Z. 5 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; two years; heart investigation.

Smear: Gram positive cocci, fairly large, mainly in

pairs and tetrads.

Colony: About 1 mm. diameter, round, flat surface, edge

slightly irregular, cream coloured, shiny, soft.

In some ways, except for properties of growth and amount of foetid odour produced, this strain resembles <u>M.asacc-harolyticus</u> which was not described in Section A. of this paper because it had not been recorded as an inhabitant of the respiratory tract. According to the key of identification of Foubert and Douglas (32) this strain falls into the species of <u>M. asaccharolyticus</u> or <u>M. aerogenes</u>, which are not differentiated. It does not conform with the description of <u>M. aerogenes</u> in Bergey's Manual in being non-haemolytic, in not changing neutral red to yellow and in not fermenting glucose. This strain is not named.

AA.8 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; two years; well baby.

Smear: Small Gram positive cocci, singly, in pairs and clusters.

Colony: About 1 mm. diameter, round, low cone becoming flatter with a flat concentric circle about the edge, opaque, cream coloured, shiny, soft.

The only difference between this strain and Z. 5 is in the size of the cocci, and the production of a small amount of

EE. 29 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; ten months; cirrhosis of the liver.

Smear: Large Gram positive cocci, mainly in masses,

some singly, and in pairs with adjacent sides

slightly flattened.

Colony: About 1 mm. diameter, colourless, transparent, with a slightly irregular edge.

Resembles M. anaerobius, except for the size of the cocci, and the acidification of sucrose. This strain is not named.

(b) Gram Negative Cocci in Masses.

Strains of small Gram negative cocci were examined according to characteristics described for the Veillonella genus in Bergey's Manual. A table of results appears in Appendix \underline{T} .

- Gelatine: 5 ml. Difco gelatin, incubated anaerobically and examined in seven days.
- Serum broth: 5 ml. broth incubated anaerobically and examined in two and seven days.
- Deep agar: Tubes of peptone agar to a height of 10 cm., melted, cooled to 45°C. and inoculated, incubated aerobically and examined daily.
- Peptone broth: 5 ml. broth incubated anaerobically and examined in two and seven days for growth and gas.
- Hydrogen sulphide: Lead acetate paper protruding through the cotton plug in peptone broth.
- Indole: 5 ml. peptone water incubated anaerobically for five days and layered with Kovac's reagent.

- Ammonia: 5 ml. peptone water incubated anaerobically for five days and layered with Nessler's reagent.
- Milk: 5 ml. Difco Litmus milk incubated anaerobically and examined in two and seven days.
- Coagulated protein: Loeffler's blood serum slants, and 5 ml.

 Difco egg meat medium incubated anaerobically for seven days.
- Neutral red dextrose broth: 5 ml. peptone broth containing 1 per cent dextrose and 0.1 per cent of a saturated solution of neutral red, incubated anaerobically and examined in two and seven days.
- Dextrose broth: 5 ml. peptone broth containing 1 per cent dextrose incubated anaerobically and examined in two and seven days.
- Nitrate reduction: 5 ml. nitrate broth, incubated anaerobically and tested in five days by the sulphanilic acid di-methylalpha-naphthylamine method.
- Fermentations: 5 ml. peptone water containing 0.5 per cent glucose, galactose, sucrose, mannitol, maltose and inulin, incubated anaerobically and examined in seven days.
- Agar slope: Peptone agar slopes incubated anaerobically and examined in two and five days.
- Haemolysis: 4 per cent human, sheep and rabbit blood agar plates incubated anaerobically and examined in two and five days.

K. 431,A. Source: Mouth.

Medium: Blood agar plate.

Subject: Male; four years; dysentery.

Smear: Small Gram negative cocci, some singly and in pairs but mostly in masses.

Colony: Pin point to 2 mm. diameter, round, raised, opaque, cream-coloured, soft.

Resembles <u>V. parvula</u> except that on the surface of blood agar plates no haemolysis is visible. It differs from <u>V. gazogenes</u> in fermenting carbohydrates, forming hydrogen sulphide, reducing nitrates and in producing a foetid odour. This strain is identified as <u>V. parvula</u>.

K. 431,B. as K. 431,A.

K. 431,C. as K. 431,A, but produces slight, irregular haemolysis on human blood agar.

K.432,A. as K. 431,A. but does not ferment sucrose and mannitol.

K.432,B. as K.431,A.

K. 72 as K. 431,A.

K. 1211 as K. 431,A.

K. 121_2 as K. 43_1 , A.

L. 101 Source: Mouth.

Medium: Blood agar plate.

<u>Subject</u>: Female; thirteen years; rheumatic fever. as K. 43₁,A. except that no gas was produced, no hydrogen sulphide formed, carbohydrates not fermented with the exception of glucose and inulin, nitrate not reduced, neutral red only partially changed in colour.

Did not survive examination.

L. 115,A. Source: As L. 101.

Medium: As L. 101.

Subject: As L. 101.

As K. 431, A. but mannitol not fermented.

L. 115, B. As L. 115 A but mannitol, sucrose not fermented.

0. 41.A. Source: Mouth.

Medium: Blood agar plate.

Subject: Female; twelve years; convalescing tuber-

culous meningitis.

As K. 431,A.

Z. 24,B. Source: Mouth.

Medium: Blood agar plate.

Subject: Male; two years; heart investigation.

As K. 431,A.

AA. 40. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; two years; well.

Smear: Small Gram negative cocci in masses.

Colony: Pin point to 1 mm. diameter, round, raised,

opaque, cream-coloured, soft.

Possesses some of the characters of both <u>V. gazogenes</u> and <u>V. parvula</u>, resembling <u>V. gazogenes var. syzgios</u> except in the production of indole. This strain is not named.

DD.3,A. Source: Mouth.

Medium: Blood agar plate.

Subject: Male; fifteen months; anaemia.

DD.17,A. As DD. 3, A.

DD.58,A. As DD. 3, A.

II.37,A. Source: Mouth

Medium: Blood agar plate.

Subject: Male; eight years; rheumatic fever.

As K. 431,A.

<u>II.37,B</u>. As <u>II</u>. 37, A.

IL.18,A. Source: Mouth

Medium: Tomato agar plate.

Subject: Female; five years; tuberculosis contact.

As K. 431,A.

LL.50,A. Source: Mouth.

Medium: Blood agar plate.

Subject: As LL.18, A.

Smear: Small Gram negative cocci in masses.

Colony: About 1 mm. diameter, round, raised, opaque,

cream-coloured, soft.

Possesses some of the characters of both <u>V. gazogenes</u> and <u>V. parvula</u>, resembling <u>V. gazogenes var. syzgios</u> except in the production of indole. It differs from strain AA. 40 in the production of hydrogen sulphide. This strain is not named.

(c) Gram Positive Cocci Tending to Form Chains.

Tests carried out were based on the differentiating characters given for the anaerobic streptococci in Bergey's Manual and Prevot's article (84). Fermentations were carried out after the methods of McDonald, Henthorne and Thompson (64)

- and Weiss and Mercado (115). A table of results appears in Appendix $\overline{\underline{I}}$.
- Gelatine: 5 ml. Difco gelatin, incubated anaerobically and examined in seven days.
- <u>Veillon's Medium</u>: Tubes of Veillon's semisolid medium to a height of 10 cm., melted, cooled to 45°C., inoculated and incubated aerobically. Examined daily.
- Martin broth: 5 ml. broth incubated anaerobically with lead acetate paper protruding through the plug, examined in two, five and seven days.
- Martin dextrose broth: 5 ml. broth containing 1 per cent dextrose, incubated anaerobically and examined in two, five and seven days.
- Indole: 5 ml. peptone water, incubated anaerobically for five days and layered with Kovac's reagent.
- Meat broth: 10 ml. thioglycollate meat medium, examined daily.
- Milk: 5 ml. Difco Litmus milk, incubated anaerobically and examined in two, five and seven days.
- Serum broth: 5 ml. broth incubated anaerobically and examined in two, five and seven days.
- Neutral red broth: 5 ml. peptone broth containing 0.1 per cent of a saturated solution of neutral red incubated anaer-obically and examined in two, five and seven days.
- Deep agar: Tubes of peptone agar filled to a height of 10 cm., melted, cooled to 45°C., inoculated, incubated aerobically, examined daily.
- Deep blood agar: As deep agar, with 5 per cent sterile oxalated human blood added at 45°C.

Egg Meat: 5 ml. Difco egg meat incubated anaerobically and examined in two, five and seven days.

Egg white: Slants of coagulated egg white incubated anaerobically and examined in two, five and seven days.

Blood broth: 5 ml. peptone broth containing 5 per cent oxalated human blood, with lead acetate paper protruding through the plug, examined in two, five and seven days.

Fresh organs: Fresh sterile guinea pig liver suspended in 5 ml.

peptone broth, with lead acetate paper protruding through
the plug, incubated anaerobically, examined in two, five
and seven days.

Fermentations: 5 ml. calf brain broth containing 1 per cent maltose, mannite, dextrose, galactose, sucrose, arabinose, dulcite, and brom thymol blue indicator, incubated anaerobically and examined in two, five and seven days.

K. 611 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; four years; dysentery.

Smear: Gram positive cocci, diplococci and short chains.

Colony: Pin point, raised, colourless.

Possesses some of the properties of Str. putridus but does not appear to be closely enough related to be identified as that species.

Q. 63 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Female; five years; investigation of tuberculosis.

Smear: Gram positive diplococci and cocci in short

chains.

Colony: About 1 mm. diameter, raised, translucent, very shiny, colourless, soft.

The properties of this strain do not appear to be those of the recognized anaerobic streptococci. It is not named.

U. 101 Source: Respiratory tract.

Medium: Blood agar plate

Subject: Male; two years; impetigo, common cold.

Smear: Very small Gram positive cocci in chains.

Did not survive transfer.

V. 10 Source: Respiratory tract

Medium: Blood agar plate

Subject: Male; seven weeks; meningocoele

Smear: Large, pleomorphic, Gram positive cocci in chains

Did not survive transfer.

Y. 9,A. Source: Mouth

Medium: Blood agar plate.

Subject: Male; nine years; rheumatic fever.

Smear: Small Gram positive cocci, singly, pairs,

chains to six cocci.

Colony: About 1 mm. diameter, round, raised, greyishwhite, moderately opaque, shiny, soft.

Possesses the characters of <u>Str. micros</u>, except in its failure to affect neutral red. It is tentatively named <u>Str. micros</u>.

BB. 3h Source: Mouth

Medium: Blood agar plate

Subject: Male; two months; malnutrition.

Smear: Gram positive cocci, singly, pairs, short chains.

Colony: About 2 mm. diameter, raised, round, flat sur-

face, colourless, shiny, soft.

Microaerophilic. It did not survive for complete examination, but as far as tested did not appear to possess the properties of the known microaerophilic streptococcus, Str. evolutus.

(d) Non-Spore-Forming Bacilli

Much of the confusion in classification of this group of anaerobes in the past has arisen from the use of different methods and media by different investigators. There have been descriptions of methods of identifying the several genera but it was impossible to determine by morphology and colonial characters into which genus the strains isolated in this study fell, especially as the reports of species in the upper respiratory tract have been of doubtful classification. Henceforth, identical tests and media were used for all these strains, both Gram positive and negative in an effort to obtain constant re-Methods used were those Lewis and Rettger (59) employed sults. in studying Gram positive and negative non-sporulating anaerobic bacilli from the intestine; and the stock medium was the glucosecysteine formula of Lewis, Bedell and Rettger (58) used in the study of the nutritive requirements of these organisms as well as in the study of intra- and extra-group relationships (53). Tables of results appear in Appendix $\overline{\underline{I}}$.

- Motility: The hanging drop method used by Lewis and Rettger was not employed, as it had been noted how Brownian movement had sometimes been mistaken for motility in some reports in the literature. Inoculations were made into the centre of a three inch glass tube in a column of semisolid glucosecysteine medium, motility being recorded by visible growth going down the centre tube and up the sides. Incubated anaerobically and examined in four days.
- Spore Test: Thioglycollate meat medium inoculated with 0.2 ml. of a seven day egg meat medium culture, boiled for 10 minutes at 100°C., incubated and examined in seven days.
- Gelatine: 5 ml. glucose-cysteine 12 per cent gelatine incubated anaerobically and examined in thirty days.
- Milk: 10 ml. whole milk containing 1 ml. per litre of a 1.6 per cent alcoholic solution of brom thymol blue, each tube containing 5 gm. iron filings incubated under atmospheric conditions and examined in eighteen days.
- Growth temperature ranges: Thioglycollate meat medium, incubated at 25°C. and at 45°C., and examined in seven days.
- Temperature resistance: 12 ml. semisolid glucose-cysteine medium inoculated with 0.1 ml. of a three day glucose-cysteine broth culture, and exposed to 60°C. for five, ten, twenty and thirty minutes, water cooled, incubated aerobically, and examined in fourteen days.
- Catalase: Glucose-cysteine agar slants, incubated anaerobically for four days and flooded with 3 per cent hydrogen peroxide.
- Indole: 5 ml. beef-cysteine broth, incubated anaerobically for twelve days and layered with 0.5 ml. Kovac's reagent.

Hydrogen sulphide: 5 ml. glucose-cysteine semisolid agar containing 0.05 per cent basic lead acetate, incubated under paraffin seal, and examined in thirty days.

Nitrate reduction: 5 ml. glucose-cysteine broth containing
0.5 per cent potassium nitrate, incubated anaerobically
for thirteen days. Tested by the sulphanilic acid dimethyl-alpha-naphthylamine method.

Acety methyl carbonil: 5 ml. glucose-cysteine broth incubated anaerobically for four days and tested with creatin and an equal volume of 40 per cent sodium hydroxide.

Fermentations: Tubes of glucose-cysteine semisolid to a height of 10 cm. containing 0.5 per cent glucose, rhamnose, mannitol, trehalose, arabinose, xylose, lactose, sucrose, maltose, salicin, incubated aerobically for fifteen days and tested by placing one drop of culture and several drops of neutral brom thymol blue solution on a slide.

1) Gram negative non-sporulating bacilli

K. 59, A. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Male; four years, dysentery.

Smear: Short, curved regular, Gram negative bacilli predominate. Rare long, regular forms.

Colony: About 1 mm. diameter, round, raised, colourless, transparent, shiny.

K. 102 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: As K. 59, A.

Smear: Pleomorphic rods, some very short, darker staining bulb at one or both poles, some long, thin filaments, some with darker staining segments.

Colony: About 1 mm. diameter, colourless, shiny, similar to a drop of water.

K. 130 Source: Mouth.

Medium: Blood agar plate.

Subject: as K. 59, A.

Smear: Thin, short, slightly curved rods, with retention of stain.

Colony: Pin point, flat, round, shiny, colourless.

L.74,A. Source: Mouth.

Medium: Blood agar plate.

Subject: Female; thirteen years; rheumatic fever.

Smear: Short, thin, curved rods, poor retention of stain.

Colony: Pin point, round, raised, shiny, colourless.

L. 75 Source: Mouth.

Medium: Blood agar plate.

Subject: as L. 74, A.

Smear: Short, curved, regular rods.

Colony: Pin point to 1 mm. diameter, round, raised,

shiny, colourless.

L.762 Source: Mouth.

Medium: Blood agar plate.

Subject: as L. 74, A.

Smear: Pleomorphic rods from very short coccoid to

short curved regular, rare long more irregular

forms.

Colony: About 1 mm. diameter, creamy, white, opaque, raised centre with concentric circle and flat edge, soft, shiny.

L.93,A. Source: Respiratory tract.

Medium: Blood agar plate

Subject: as L. 74, A.

Smear: Very tiny Gram negative rods.

Colony: About 1 mm. diameter, round, raised, greyish, soft, shiny.

L.1022 Source: Mouth.

Medium: Blood agar plate.

Subject: as L. 74, A.

Smear: Very tiny bacilli and coccobacilli.

Colony: Pin point round, raised, opaque, white, slightly haemolytic.

0. 35 Source: Mouth.

Medium: Blood agar plate.

<u>Subject</u>: Twelve year old female; convalescing tuberculous meningitis.

Smear: Small, short, curved rods, poor retention of stain.

Colony: Pin point, round, raised, whitish.

0. 37 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 35.

Smear: Small thin curving rods, with irregularities in thickness and staining; rare longer forms with irregular shape.

Colony: Pin point to 1 mm. diameter, white, opaque, raised, round.

0. 39. Source: Mouth.

Medium: Blood agar plate.

Subject: as 0.35.

Smear: Short and thin bacilli, slightly curving, some diplococci.

Colony: One to two mm. diameter, round, raised, colour-less, shiny, translucent.

0. 51 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0.35.

Smear: Very short, slightly curved rods.

Colony: Pin point to about one mm. diameter, round, raised, opaque white, very slightly haemolytic.

This strain did not survive transfer.

0. 72 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 35.

Smear: Pleomorphic bacilli with darker staining granules, slightly thicker at one pole, some tapering at one pole, variable in length, some with apparent pseudobranching at one pole.

Colony: About 2 mm. diameter, round, raised, with flat edge, opaque, cream, dull.

Microaerophilic.

0. 90 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 35.

Smear: Short, curved, irregular rods with Gram positive

granules.

Colony: Pin point to 1 mm. diameter, round, raised, greyish white, soft, dull.

Microaerophilic.

Q. 1 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Five year old female; under investigation for tuberculosis.

Smear: Pleomorphic bacilli, short thin straight,
longer, curved, some thicker at one pole, rare
short thick ovoid form.

Colony: Pin point to 1 mm. diameter, round, raised, shiny, colourless, translucent.

Q. 2 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 1.

Smear: Thin bacilli from coccobacilli to long, curved.

Colony: Pin point, round, raised, whitish.

Q. 13 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 1.

Smear: Bacilli tending to form curving filaments, some pseudobranching, some Gram positive granules, some very short and irregular.

Colony: About ½ mm. diameter, round, raised, with flat surface, greyish white.

Microaerophilic.

Q. 84 Source: Mouth

Medium: Blood agar plate.

Subject: as Q. 1.

Smear: Thin, short, regular bacilli, with tapered end.

Colony: About 2 mm. diameter, flat with an irregular

edge, raised to a point in the centre, greyish

white.

Did not survive transfer.

Q. 100 Source: Mouth

Medium: Blood agar plate.

Subject: as Q. 1.

Smear: Short, curved bacilli.

Colony: About ½ mm. diameter, round, raised, black

pigment, dry and adhering to medium.

Did not survive transfer.

R. 11 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Ten year old male with pleurisy.

Smear: Small bacilli from coccoid to short, curved,

irregular.

Colony: About ½ mm. diameter, flat, round, colourless,

translucent.

R. 23 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as R. 11.

Smear: Very irregular short bacilli, some like diplo-

cocci, rare tangled short filament.

Colony: Pin point colourless.

Did not survive transfer.

- /3 -

R. 47 Source: Mouth.

Medium: Blood agar plate.

Subject: as R. 11

Smear: Thin straight bacilli, rare V. and Y. forms.

Colony: Pin point colonies with dry filamentous edge.

Did not survive transfer.

R. 78 Source: Mouth.

Medium: Blood agar plate.

Subject: as R. 11.

Smear: Short, curved bacilli.

Colony: About ½ mm. diameter, round, raised, black

pigment, adhere to medium.

Did not survive transfer.

T. 107 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Ten year old male, with tuberculous gland in neck.

Smear: Bacilli from very short, to moderate length,

slight curving.

Colony: Pin point to ½ mm. diameter, round, raised,

colourless.

U. 103 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Two year old male, with impetigo, common cold.

Smear: Tiny coccobacilli and short rods.

Colony: Pin point, colourless.

Did not survive transfer.

U. 110 Source: Mouth

Medium: Blood agar plate.

Subject: as U. 103.

Smear: Very small, short, thin bacilli

Colony: Pin point, colourless, flat.

U.1101 Source: Mouth

Medium: Blood agar plate

Subject: as U. 103.

Smear: Mainly short bacilli and diplobacilli, rare

longer, irregular forms.

Colony: About 1 mm. diameter, round, raised, shiny,

creamy white, opaque.

U. 126 Source: Mouth.

Medium: Blood agar plate.

Subject: as U. 103.

Smear: Bacilli, coccobacilli to long straight and

surved thin rods.

Colony: About ½ mm. diameter, round, raised, with

flat top, colourless, shiny.

U. 129 Source: Mouth.

Medium: Blood agar plate.

Subject: as U. 103.

Smear: Pleomorphic bacilli, curved, thick and very

thin, pseudobranching, some thicker segments.

Colony: Pin point, colourless, raised.

V. 44 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Seven week male, with meningocoele

Smear: Thin, slightly curved bacilli and filaments.

Did not survive transfer.

W. 35 Source: Mouth.

Medium: Blood agar plate.

Subject: Nine year old male with tuberculous pleurisy.

Smear: Curving bacilli, mainly short, rare long forms.

Colony: Pin point, round, raised, colourless.

W. 46 Source: Mouth.

Medium: Potato.

Subject: as W. 35.

Smear: Faintly staining bacilli, coccobacilli and

longer curved, slightly irregular.

Colony: About & mm. diameter, colourless, depressed

in the surface of medium.

Did not survive transfer.

X. 61 Source: Mouth.

Medium: Blood agar plate.

Subject: Eleven year old female, with bronchiectasis.

Smear: Straight, thin bacilli of varying length.

Colony: About \frac{1}{2} mm. moderately flat, colourless, shiny.

X. 79 Source: Mouth.

Medium: Blood agar plate.

Subject: as X. 61.

Smear: Mainly very small, slightly curved bacilli.

Colony: Pin point, round, raised, colourless.

X. 86 Source: Mouth.

Medium: Potato.

Subject: as X. 61.

Smear: Slightly curving, filamentous bacilli.

Did not survive transfer.

Z. 50 Source: Mouth.

Medium: Blood agar plate.

Subject: 2 year old male, undergoing heart investigation.

Smear: Tangled filaments and curved bacilli.

Did not survive transfer.

DD. 7 Source: Mouth.

Medium: Blood agar plate.

Subject: Fifteen month male with anaemia.

Smear: Very short, curved, and slightly irregular bacilli.

Colony: Pin point, flat, shiny, whitish.

LL.25,B. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Five year old female, well.

Smear: Thin, short, curved bacilli.

Colony: Pin point, colourless.

LL.76, B. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as LL. 25, B.

Smear: Thin, very short, slightly curved bacilli.

Colony: Pin point, colourless.

LL.106 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as LL. 25, B.

Smear: Mainly short, slightly irregular bacilli, rare

long, curving, irregular.

Colony: About ½ mm. diameter, round, raised, black

pigment, dry, adhered to medium.

Did not survive transfer in several media.

2) Gram positive non-sporulating bacilli

F.54,B1 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Thirteen month male, with Staphylococcus pyogenes lesions.

Smear: Short curved, regular bacilli in diphtheroid arrangement.

Colony: About 1 mm. diameter, round, raised, shiny, greyish, becoming opaque white.

Microaerophilic; haemolytic when aerobic.

F.622 Source: Respiratory tract.

Medium: Blood agar plate

Subject: as F. 54, B1.

Smear: Short, thin bacilli, with bulbs, Y, comma forms, some lighter staining segments, rare streptococcus-like forms.

Colony: Pin point, round, raised, greyish, becoming white opaque.

F.623 Source: respiratory tract.

Medium: Blood agar plate.

Subject: as F. 54, B1.

Smear: Short, thin, curved bacilli, diphtheroid aspect, some more deeply staining at one pole.

<u>Colony</u>: Pin point, round, raised, greyish, becoming white opaque.

F.84,A. Source: respiratory tract.

Medium: Blood agar plate.

Subject: as F. 54, B1.

Smear: Short, curved, fairly thick bacilli, some diplococci, some coccobacilli.

Colony: Pin point, round, raised, greyish becoming white, opaque, haemolytic.

G.911 A. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Twelve year old female, rheumatic fever.

Smear: Very pleomorphic bacilli; branching, irregular shape, large bulbs, Gram negative segments.

Colony: Colourless pin point, some very thin bacilli, some very thick irregular bodies.

G.912 A. Source: respiratory tract.

Medium: Blood agar plate.

Subject: as G. 911, A.

Smear: Thick pleomorphic bacilli showing bulbs, branching, X and Y forms, irregular bodies.

Colony: Pin point to ½ mm. diameter, round, raised, greyish, slightly alpha haemolytic.

G.99,A. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 911, A.

Smear: Short thin bacilli, diphtheroid aspect, some coccobacilli.

Colony: Pin point round raised becoming opaque white.

G.99,B. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 911, A.

Smear: Thick and thin pleomorphic bacilli, showing irregular bulbs, branching, varying in length.

Colony: About ½ mm. diameter, round, raised, greyish, shiny, opaque.

J.4,A1 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Three year old male, bronchial asthma.

Smear: Curved, branching bacilli, X & Y forms,

varying length.

Colony: About one mm. diameter, round, raised,

greyish becoming brownish.

Microaerophilic.

J.4,C1 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as J.4,A1.

Smear: Short, curved, regular bacilli.

Colony: About 1 mm. diameter, round, raised, greyish

becoming opaque white, haemolytic.

J.4,D2 Source: respiratory tract.

Medium: Blood agar plate.

Subject: as J.4,A1.

Smear: Short thick bacilli, with irregular pro-

jections, branching.

Colony: About $\frac{1}{2}$ mm. diameter, round, raised, greyish-

white, granular.

Microaerophilic.

J. 62 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as J.4,A1.

Smear: Mainly very short coccobacillary forms, some

short curved bacilli and rare length, curved

forms.

Colony: Pin point, round, raised, greyish.

K. 37. Source: Mouth.

Medium: Blood agar plate.

Subject: Four year old male; dysentery.

Smear: Coccobacilli, short, curved and straight

rods, with irregular outline, some very thin

others with thick sections.

Colony: Pin point, round raised, opaque white with

irregular areas of haemolysis.

K. 501 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as K. 37.

Smear: Short straight to slightly curved bacilli,

with some thickenings.

Colony: About $\frac{1}{2}$ mm. diameter, round, raised, with

flat surface, shiny, whitish.

Microaerophilic.

K.502 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as K. 37.

Smear: Bacilli of varying length, slightly curved,

some irregular thickenings.

Colony: About 1 mm. diameter, round, raised, with

surface fairly flat, shiny, whitish.

Microaerophilic.

K. 97 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as K. 37.

Smear: Long bacilli, very curving, Gram negative segments.

Did not survive transfer.

K.1191 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as K. 37.

Smear: Pleomorphic bacilli, much variation in length,

surved, irregular thick parts, possible branching.

Colony: Pin point round, raised with flat surface,

colourless, shiny.

L. 109 Source: Mouth.

Medium: Blood agar plate.

Subject: Thirteen year old female, rheumatic fever.

Smear: Very short and moderately long, regular bacilli.

Colony: About 1 mm. diameter with irregular edge and

surface, opaque white, soft, very haemolytic.

0. 3. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Twelve year old female, convalescing tuber-

culous meningitis.

Smear: Short bacilli, rare coccoid and long curved

forms.

Colony: About 1 mm. diameter, round, raised, opaque

white, soft.

0. 60 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Short, curved, regular bacilli.

Colony: About 1 mm. diameter, round, raised,

opaque, white, soft.

Microaerophilic.

0. 71 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Large bacilli, irregular thick parts, Gram

negative segments, branching, rare strepto-

coccus-like forms.

Colony: 1-2 mm. diameter, round, raised, opaque,

cream, granular.

Microaerophilic.

0. 91 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Very long bacilli, darker staining granules.

branching, irregular bodies. Not acid fast.

Colony: About 1 mm. diameter, round, raised, opaque,

white with a filamentous edge and surface.

0.95.A. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Bacilli, thin and tangled, and filaments.

Colony: About 1 mm. diameter, flat, round, greyish, shiny.

Microaerophilic.

0.95,B. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Pleomorphic bacilli, thick parts, bulbs, seg-

ments, Gram negative with Gram positive granules.

Colony: About ½ mm. diameter, flat, round, greyish,

granular surface.

0. 100 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Short, thick bacilli with square ends,

rare thin, slightly curved forms.

Colony: 1-2 mm. diameter, flat, chiny, with a dull

irregular edge.

Microaerophilic.

<u>0. 101</u> Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as 0.3.

Smear: Bacilli of varying length, tending to be

filamentous, branching, irregular shapes

and chains.

Colony: About ½ mm. diameter, flat, shiny, colourless,

with an irregular edge.

Microaerophilic.

0. 105 Source: Mouth.

Medium: Blood agar plate.

Subject: as 0. 3.

Smear: Thick bacilli with round ends, varying lengths,

surving, some are thin with one thick pole.

Colony: 1-2 mm. diameter, round, raised with flat

surface, granular.

Q. 7 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Five year old female, investigation of tuberculosis

Smear: Thin, irregular, short, curving bacilli.

Colony: Pin point, round, raised, white.

Q. 20 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 7.

Smear: Short, curved bacilli tending to become very

short or longer and irregular like cocci in

chains.

Colony: About 2 mm. diameter, round, raised, greyish

white, soft, shiny.

Q. 27 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 7.

Smear: Very short, thick, curved bacilli, become more

irregular, coccoid, some Gram negative segments.

Colony: 1-2 mm. diameter, round, raised, greyish,

white, soft.

Microaerophilic

Q.272A. Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 7.

Smear: Short, curved bacilli tending to form clusters

of heavy irregular forms, very pleomorphic,

some Gram negative segments.

Colony: Pin point colourless.

Q. 29 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as Q. 7.

Smear: Short, thick bacilli, some thickening at one

pole, some appear Gram negative with Gram

positive granules.

Colony: 1-2 cm.diameter, round, raised, opaque, cream,

granular.

S. 761 Source: Mouth.

Medium: Blood agar plate.

Subject: Fifteen month female, tonsillitis.

Smear: Short, curved, regular bacilli, diphtheroid aspect.

Colony: About 1 mm. diameter, round, raised, greyish, soft.

S. 762 Source: Mouth.

Medium: Blood agar plate.

Subject: as S. 761.

Smear: Short bacilli in chains up to eighteen, isolated

forms longer.

Colony: 1-2 mm. diameter, round, flat, grey, dull,

slightly granular.

T. 46 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: 10 year old male; tuberculous gland in neck.

Smear: Curving, irregular filaments, some like

irregular thick bacilli with thin short

curving, Gram negative projection.

T. 111 Source: Mouth

Medium: Blood agar plate.

Subject: as T. 46.

Smear: Thick regular bacilli of varying lengths,

slightly curving.

Colony: 1-2 mm. round raised, irregular edge,

transparent, colourless, shiny.

Microaerophilic.

U. 107 Source: Mouth.

Medium: Blood agar plate.

Subject: Two year old male; impetigo, common cold.

Smear: Short, straight or slightly curving bacilli.

Colony: Pin point, round, raised, opaque, white.

U. 123 Source: Mouth.

Medium: Blood agar plate.

Subject: as U. 107.

Smear: Bacilli from coccoid to short thick straight.

Colony: About 1 mm. diameter, round, raised, greyish.

U. 127 Source: Mouth.

Medium: Blood agar plate.

Subject: as U. 107.

Smear: Bacilli from coccoid to short curved, rare

irregular forms.

Colony: Pin point, round, raised, greyish.

W. 19 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Nine year old male, tuberculous pleurisy.

Smear: Very short thin, slightly irregular bacilli.

Colony: About 1 mm. diameter, round, slightly raised,

flat surface, colourless, shiny.

W. 45 Source: Mouth.

Medium: Blood agar plate.

Subject: as W. 19.

Smear: Curved bacilli of varying length, regular.

Colony: About ½ mm. diameter, round, raised, cream,

opaque.

X. 20 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Eleven year old female, bronchiectasis.

Smear: Short bacilli; straight, curved, some tapered,

some with thickening at one pole.

Colony: About 1 mm. diameter, round, raised, greyish-

white, opaque.

X. 38,A. Source: Mouth.

Medium: Blood agar plate.

Subject: as X. 20.

Smear: Thin curved bacilli, some short, some fila-

ments, rare bulbs, Gram negative segments,

some evidence of branching.

Colony: Pin point, round, raised, opaque, white.

X. 42 Source: Mouth.

Medium: Blood agar plate.

Subject: as X. 20.

Smear: Bacilli of varying lengths, some filaments,

evidence of branching, some filaments.

Colony: Pin point, round, raised, opaque, greyish white.

X. 711 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as X. 20.

Smear: Short straight thin regular bacilli.

Colony: Pin point, round, raised, whitish.

Y. 3 Source: Mouth.

Medium: Blood agar plate.

Subject: Nine year old male; rheumatic fever.

Smear: Very short thin curved regular bacilli.

Colony: About 1 mm. diameter, round, raised, opaque, white.

Y. 17 Source: Mouth.

Medium: Blood agar plate.

Subject: as Y. 3.

Smear: Short thick curved bacilli, some large coccoid.

bodies, irregular thickenings.

Colony: About ½ mm. diameter, round, raised, greyish.

CC. 11 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Eight day female; anaemia.

Smear: Coccobacilli and very short rods.

Did not survive transfer.

EE. 5 Source: Mouth.

Medium: Blood agar plate.

Subject: Ten month male; cirhossis of the liver.

Smear: Short curved bacilli to moderate lengths,

rare long forms like cocci in chains,

mainly regular.

Colony: About ½ mm. diameter, round, raised, with

flat surface, colourless, shiny.

II. 16 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Eight year old female; rheumatic fever.

Smear: Thick, curved bacilli, some tapered.

Did not survive transfer.

KK. 29 Source: Mouth.

Medium: Blood agar plate.

Subject: Ten year old male; infectious hepatitis.

Smear: Thin bacilli showing branching, and large bulbs

at one or both poles. Some Gram negative segments.

Colony: Pin point, round, raised, opaque, cream.

Microaerophilic.

KK. 40 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: as KK. 29.

Smear: Short curved regular bacilli and longer

curving irregular forms.

Colony: About 1 mm. diameter, round, raised, surface

flat, greyish white, shiny.

Microaerophilic.

IL. 99 Source: Respiratory tract.

Medium: Blood agar plate.

Subject: Five year old female; well.

Smear: Curved, branching clubbed bacilli.

Did not survive transfer.

MM. 36 Source: Mouth

Medium: Blood agar plate.

Subject: Eleven year old male; pleurisy.

Smear: Small short thin curved regular bacilli.

Colony: Pin point, round, raised, opaque, white.

(e) Gram Positive Spore-forming Bacilli.

Gram positive spore-forming bacilli were isolated from two subjects, R. and S. They were examined according to the criteria presented in Bergey's Manual for the identification of Clostridia, and yielded identical results.

Smear: Gram positive bacilli with a subterminal to central oval spore which swelled the bacillus slightly. Some long chain formation, many pairs. Nigrosin stains confirmed the presence of spores.

- Colony: Two types; small, colourless, water drop-like and a whitish, dried looking colony up to 4 mm. diameter with an irregular edge. Either colony when replated gave rise to both colony types, and smears from both showed both straight rods with round ends showing no spore, and oval ones containing a large spore.
- Spore Test: Thioglycollate meat cultures boiled at 100°C. for ten minutes and inoculated into fresh media yielded growth.

 Motility was observed in semisolid peptone agar.
- Coagulated albumen and coagulated rabbit serum were not attacked in two weeks' anaerobic incubation.
- Difco Litmus milk was neither acidified nor coagulated in two weeks' anaerobic incubation.
- Hiss serum 0.5 per cent solutions of dextrose, mannitol, starch, lactose, sucrose, salicin and adonitol were not fermented in eighteen days anaerobic incubation.
- Colonies on plain peptone agar were scanty and colourless when incubated anaerobically.
- Colonies in deep peptone agar were tiny, punctiform to lenticular.
- Indole was formed in peptone water incubated anaerobically and tested in seven days with Kovac's reagent.
- No gas was observed in any of the above media, nor in deep dextrose agar cultures.
- The strains were not pathogenic for mice when inoculated intramuscularly in calcium chloride solution.
- This species did not conform with any of the species listed in the Manual and was thought to be a contaminant.

PART III A REVIEW OF FINDINGS

- F. Male; 13 months; pyogenic lesions on face, legs.
 - 3 strains anaerobic non-spore-forming Gram positive bacilli.
 - l strain microaerophilic non-spore-forming Gram positive bacilli.
- G. Female; 12 years; rheumatic fever.

4 strains Gram positive anaerobic non-spore-forming bacilli.

H. Male; 2 months; dysentery.

No anaerobes isolated.

I. Male; 8 years; rheumatic fever.

No anaerobes isolated.

J. Male; 3 years; bronchial asthma.

Two strains Gram positive anaerobic non-spore-forming bacilli.

Two strains Gram positive microaerophilic non-sporeforming bacilli.

K. Male; 4 years; dysentery.

One strain Veillonella parvula (7 cultures).

One strain anaerobic streptococcus.

Three strains Gram negative anaerobic non-spore-forming bacilli.

Two strains Gram positive anaerobic non-spore-forming bacilli.

Two strains Gram positive microaerophilic non-sporeforming bacilli.

One strain Gram positive bacillus, which did not survive examination.

L. Female; 13 years; rheumatic fever.

One strain Veillonella parvula (2 cultures).

Five strains Gram negative anaerobic non-spore-forming bacilli.

One strain Gram positive anaerobic non-spore-forming bacilli.

O. Female; 12 years; convalescing tuberculous meningitis.

One strain <u>Veillonella parvula</u>.

Three strains Gram negative anaerobic non-spore-forming bacilli.

One strain Gram negative microaerophilic non-spore-forming bacilli.

Two strains Gram positive anaerobic non-spore-forming bacilli.

Six strains Gram positive microaerophilic non-sporeforming bacilli.

P. Male; 1 month; diarrhoea.

One strain Micrococcus.

One strain Gram positive anaerobic cocci in clusters, which did not survive.

Q. Female; 5 years; investigation of tuberculosis. anaerobic

One strain/streptococcus.

Two strains Gram negative anaerobic non-spore-forming bacilli.

One strain Gram negative microaerophilic non-spore-forming bacilli.

Two strains Gram negative anaerobic non spore-forming bacilli, which did not survive, one tentatively identified as B. melaninogenicum.

Four strains Gram positive anaerobic non-spore-forming bacilli.

One strain Gram positive microaerophilic non-spore-forming bacilli.

R. Male; ten years; pleurisy.

One strain Gram negative anaerobic non-spore-forming bacilli.

Three strains Gram negative anaerobic non-spore-forming bacilli, which did not survive transfer. One tentatively identified as B. melaninogenicum.

One strain Gram positive anaerobic spore-forming bacilli.

S. Female; fifteen months; tonsillitis.

Two strains Gram positive anaerobic non-spore-forming bacilli.

One strain Gram positive anaerobic spore-forming bacilli.

T. Male; ten years; tuberculous gland in neck.

One strain Gram negative anaerobic non-spore-forming bacilli.

One strain Gram positive anaerobic non-spore-forming bacilli.

One strain Gram positive microaerophilic non-spore-forming bacilli.

U. Male; two years; impetigo, common cold.

One strain Gram positive cocci in chains which did not survive transfer.

Four strains Gram negative anaerobic non-spore-forming bacilli.

One strain Gram negative anaerobic bacilli which did not survive transfer.

Three strains Gram positive anaerobic non-spore-forming bacilli.

V. Male; seven weeks; meningocoele.

One strain Gram positive cocci in clusters which did not survive transfer.

One strain Gram positive cocci in chains which did not survive transfer.

One strain Gram negative bacilli which did not survive transfer.

W. Male; nine years, tuberculous pleurisy.

One strain Gram negative anaerobic non-spore-forming bacilli.

One strain Gram negative bacilli which did not survive transfer.

Two strains Gram positive anaerobic non-spore-forming bacilli.

X. Female; eleven years; bronchiectasis.

Two strains Gram negative non-spore-forming anaerobic bacilli.

One strain Gram negative bacilli which did not survive transfer.

Four strains Gram positive non-spore-forming anaerobic bacilli.

Y. Male; nine years; rheumatic fever.

One strain anaerobic streptococcus, tentatively identified as Str. micros.

Two strains Gram negative non-spore-forming anaerobic bacilli.

Z. Male; two years; heart investigation.

One strain anaerobic micrococcus.

One strain Veillonella parvula.

One strain Gram negative bacilli which did not survive.

AA. Male; 2 years; well.

One strain micrococcus.

One strain Veillonella.

BB. Male; 2 months; malnutrition.

One strain microaerophilic streptococcus.

CC. Female; 8 days; anaemia.

One strain Gram positive bacilli which did not survive.

DD. Male; 15 months; anaemia.

One strain Veillonella parvula (3 cultures).

EE. Male; 10 months; cirhossis of liver.

One strain micrococcus.

One strain Gram positive non-spore-forming anaerobic bacilli.

GG. Male; 2 months; diarrhoea.

No anaerobes isolated.

HH. Female; 4 years; coryza, cough.

No anaerobes isolated.

II. Male; 8 years; rheumatic fever.

One strain Veillonella parvula (2 cultures)

One strain Gram positive bacilli which did not survive.

KK. Male; 10 years; infectious hepatitis.

Two strains microaerophilic Gram positive bacilli.

LL. Female; 5 years; well, tuberculosis contact.

One strain Veillonella parvula.

One strain Veillonella.

Two strains Gram negative anaerobic non-spore-forming bacilli.

One strain Gram negative bacilli which did not survive transfer, tentatively identified as B. melaninogenicum. One strain Gram positive bacilli which did not survive.

MM. Male; ll years; pleurisy.

One strain Gram positive anaerobic non-spore-forming bacilli.

PART IV DISCUSSION

The information received from a survey of the literature, upon which to base this study, was, to a great extent, derived from work carried out on bacterial species recovered from parts of the body other than the respiratory tract. There were, of course, some notable exceptions in the work by Prevot on the anaerobic cocci, and in the several studies on the fusiform bacilli. The studies carried out on children have been very few in number, limited to one or two surveys of buccalpharyngeal flora, and reports of species from the upper respiratory tract have been almost entirely incidental to other types Methods and media used varied with almost every of studies. For this reason, it being impractical to employ investigator. all methods and media mentioned, techniques had to be found which would be suitable for a wide range of bacterial species. Attempts at identification were in some cases, quite clear-cut, species and genera being fairly well established, although it is thought that many species may not yet have been described and In the case of the large group of anaerobic non-sporenamed. forming bacilli, it was necessary to attempt identifications on

the basis of descriptions of bacterial species recovered largely from the intestinal tract. Descriptions of species actually recovered from the respiratory tract have yielded very scanty information or only morphological reports, so correlation of strains isolated in this study with past work is not very satisfactory.

In all probability, some of the anaerobic species inhabiting the respiratory tract were not recovered, for many appear to require specialized media which may not have been included here. As has been previously mentioned, no attempts were made to isolate species of the genus Dialister or Spirochaetales. Furthermore, several strains of both cocci and bacilli did not survive transfer because in all probability the stock medium of thioglycollate meat did not supply the necessary nutritive requirements for these strains. It has been noted previously in this paper, that, in the case of the Bacteroides and Fusobacterium, isolation and maintenance have been found by many workers to be difficult, and in the case of Actinomyces alternate transfers to different media were necessary to main-This may be true of several bacterial types also. tain growth.

Much more investigation of the nutritive requirements of anaerobic species is necessary in order to obtain more accurate and complete results in further studies of this kind.

Morphological examinations were carried out on many of the specimens in order to supplement the information received from cultural and biochemical examinations. Brooke's strain was done on both the mouth and respiratory tract of material from all patients from U. on.

The results of these were not included in the review of findings as all showed a mixed flora, the significance of which could not be determined, as much of it, no doubt, was a picture of aerobic species. The only points of interest are In the eight day old infant (CC) the flora the following. was limited to rare Gram positive and Gram negative regular bacilli. This picture was supported by cultural studies. Children only a few weeks old (V, BB, GG) showed flora of mixed bacterial types, but more limited than that of the older children, material from most of whom showed a very varied morphological picture. It is of interest to note that of the six cases where spirochaetes were seen in smear, one was from the mouth of an eight week old infant (BB). The others were from the throat of a ten year old (KK), throat of an eight year old (II), mouth of a four year old (HH), mouth of a fifteen month old (DD) and mouth of an eleven year old (X). Two types of spirochaetal forms were seen in the last. No attempt was made to identify them as this method of examination was not considered adequate.

It would be well worth while to carry out further studies of spirochaetes and their occurrence in the mouths of children.

Six strains of anaerobic micrococci were recovered from five subjects, four of which survived transfer to undergo biochemical examination. All were isolated on blood agar. They were recovered from both the mouth and respiratory tract, from different diseases and from different age groups. None of the strains was named.

Seven subjects yielded <u>Veillonella parvula</u>. One of these, and an eighth child harboured unnamed strains of Veillonella. Age, sex, and history varied, and the strains were recovered from both the mouth and respiratory tract.

Anaerobic streptococci were recovered from six patients. Four were examined biochemically and, as a result, one was tentatively identified as Str.micros. Again there was no correlation between the findings and age, sex, disease or source.

The significance of the presence of the anaerobic cocci in the upper respiratory tract cannot be determined from this introductory study. A larger series might yield more significant results. One would expect to find anaerobic streptococci in the throats of infants, as they apparently inhabit the vagina in large numbers (102). In this study the streptococci were found in older children except in the cases of a seven week old and a two month old infant. Very few of the cocci were recognized to be previously described species. Further investigation may yield several more species which to date have not been recognized.

negative non-spore-forming bacilli as any one of the recognized species of either Bacteroides or Fusobacterium met with little success. Twenty-eight strains were examined biochemically. The results were extremely varied but one cannot believe that each organism belongs to a different species. An attempt to separate the strains into groups has been only partially successful. One group could include the straight or slightly

curved bacilli of varying length but without irregularities in shape forming unpigmented colonies. This separation was supported by the facts that none of these strains liquefied gelatine, none acidified milk, all grew at 25°C., none at 45°C. except W. 35, none reduced nitrates except X. 61. and all were strict anaerobes and non-branching. The results of all other tests were too variable to be of any significance. This group included eleven strains from eight subjects (K. 59 A; K. 130; L. 74 A; L. 75; O. 39; T. 107; U. 126; W. 35; X. 61; X. 79; LL. 25, B). The remaining seventeen strains tested included a heterogeneous group of organisms which could not be separated satisfactorily on the basis of tests carried out. Whether some of the cultures from one patient subjected to examination might be in reality the same strain producing varying results or whether they be all separate strains cannot be determined by these criteria. The few strains producing black pigment may have been Bacteroides melaninogenicum. This assumption was based on morphology and production of the striking pigment only as all strains did not survive transfer.

The Gram positive non-spore-forming bacilli could not be identified with any more success than the Gram negative ones. Fifty strains were examined, no two yielding identical results. At best, the strains could be grouped into anaerobic or microaerophilic bacilli, branching or non-branching, pleomorphic with bulbs and irregularities or straight or slightly curved regular bacilli. These groups did not appear to be adequately substantiated by biochemical tests.

The Gram positive and Gram negative non-sporulating

bacilli were isolated from both the respiratory tract and mouth, from all ages and from a variety of diseases. Their significance cannot be determined, although they were the most common types of anaerobic bacteria found in the upper respiratory tract. Gram negative bacilli were isolated from thirteen and Gram positive bacilli from ninteen of the thirty children.

The only hope of classification of these nonsporulating bacilli would seem to lie in serological examinations. In this group of seventy-eight strains which varied
so greatly morphologically, culturally and biochemically, some
relationships might be obtained by serological methods.

This has been a preliminary study. It has shown the presence of several groups of anaerobic bacteria in the upper respiratory tract in children. As attempts to identify strains met with little success, and the series was small and the results varied, it was not possible to associate any bacterial types with source, age, sex, or disease.

media. The validity of several species, particularly of the cocci, which have not been previously described may be confirmed. The anaerobic non-spore-forming bacilli, always a subject of confusion, require of course much more investigation. It would be of interest to study these strains by serological methods. Investigation of the pathogenicity of strains might also aid in showing the significance of the flora.

PART V SUMMARY

Surveys of the anaerobic flora of the upper respiratory tract have been few in number, particularly in children. Much of the information concerning anaerobic bacterial species inhabiting the buccal-pharyngeal cavity has been obtained from studies of organisms from other parts of the body. The literature has shown that classification of the anaerobic bacteria is still in a state of indecision.

A study has been made of the anaerobic flora of the nose, throat, nasopharynx and mouth in thirty children ranging in age from eight days to thirteen years, hospitalized for a variety of diseases at the Children's Memorial Hospital.

Morphological, cultural and biochemical examinations were carried out on strains of the several bacterial groups recovered.

Anaerobic Micrococci were isolated from five subjects.

None was named. Seven children yielded <u>Veillonella parvula</u>.

One of these, and an eighth child harboured an unnamed Veillonella. Anaerobic streptococci were recovered from six patients.

One was tentatively identified as <u>Str. micros</u>.

from thirteen children, twenty-eight strains being subjected to biochemical examination. Fifty strains of Gram positive non-spore-forming bacilli isolated from ninteen subjects were tested. Both groups yielded such diverse results that they could not be identified according to examinations carried out.

Spirochaetes were seen by morphological examination of material from six children.

No correlation could be made between bacterial types and source, age, sex and disease.

Further work, including a larger series and studies on pathogenicity may establish significant relations between bacteria and disease, and the flora of the normal upper respiratory tract in children at different ages. Serological examinations may help to determine a more satisfactory classification of the non-spore-forming bacilli.

a) Gram positive cocci in clusters

	P. 60	Z. 5	AA. 8	五五。29
Gelatine		3	2	1
Deep agar	tiny round lenticular colonies no gas	small colonies lenticular broken by gas	small colonies lenticular	small colonies lenticular no gas
Serum agar	1 54	small colonies lenticular broken by gas	small colonies lenticular broken by gas	1() ***
Deep dextrose agar	no gas	gas bubbles	gas bubbles	no gas
al red	round raised	small, lenticular	small, lenticular	round, colourless
serum agar	surface, lenti-	colonies, changed	colonies, changed	surface, Lenticular
	No colour change			colour change
	shange	no change		slightly darkened
blood broth	H2S Slightly \neq	Н255 -	HZS SIIgntly 7	HZS SIIgntly 7
Dextrose	42	fine floccular	fine floccular	slight turbidity
serum broth	white sediment	sediment	sediment	sediment
Nitrate reduction	f	<i>f</i>	<i>f</i>	1
Peptone water	turbid, indole	scanty sediment		turbid,
with serum	2	indole \neq		indole 🕶
	slightly acid	slightly acid	slightly acid	no change
Loeffler's				
blood serum	not digested	not digested	not digested	not digested
Neutral red broth	no change	no change	changed to orange	no change
Coagulated		•		
egg white	not digested	ا. ا	not digested	- 1
Coagulated serum	not digested	not digested	not digested	not digested
Lactose	acid	3	1	8
Sucrose	acid	ŧ	-	slight acid
Dextrose	acid clot	1		slight acid
Maltose			1	•
Mannitol		•		3
Starch	acid clot		1	1
Odour	not foetid	slightly foetid	slightly foetid	slightly foetid

b) Gram negative cocci

	K. 431 A	K. 431B	К. 431 С	K. 432 A
Agar slope colonies	pin point colourless	pin point colourless	pin point colourless	pin point colourless
Odour	slight foetid	slight foetid	slight foetid	slight foetid
Hemolysis			slight	
Hemolvsis			TITEBATAT	
sheep blood	1	1	1	1
Hemolysis rabbit blood	t v	1	ſ	1
Gelatine liquefaction	1	1	1	1
Serum broth	heavy turbidity sediment	heavy turbidity sediment	heavy turbidity sediment	heavy turbidity sediment
Deep agar	broken by gas col.small lentic.	bro col	broken by gas	broken by gas
Growth in	116 3			turbid
peptone broth	gas			gas
Indole	, t	slight /	slight /	slight /
•~	slight	slight	slight	slight
Ammonia	T TOGG UTAIL	I TOCCULAT	ITOCCULAR	TTOCCULAR
Milk	no c hange	no change	no change	no change
Loeffler Egg meat	no	1	1	1
Hydrogen sulphide	f	<i>f</i>	f	
L	changed	changed	changed	changed to
dextrose broth	HІ	\dashv	⊣ I	yellow
O I	turbid sediment	turbid sediment	turbid sediment	turbid sediment
Nitrate reduction	ı	+	7	
Glucose		slight acid	acid	slight acid
Galactose	- 1	slight acid	acid	slight acid
Sucrose	slight acid	slight acid	acid	
Mannitol	ı		acid	
Maltose	- 1		acid	slight acid
Inulin	slight acid	slight acid	acid	

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<u>م</u>
cocci
negative
Gram
Q

	K. 432 B	K. 72	K. 121 ₁	K. 1212
Agar slope colonies	pin point colourless	pin point colourless	pin point colourless	pin point colourless
Odour	slight foetid	slight foetid	slight foetid	slight foetid
Hemolysis human blood	ı	1	1	.1
Hemolysis sheep blood	1		1	1
	1	1	1	1
Gelatine liquefaction	1	1	1	1
Serum broth	heavy turbidity sediment	heavy turbidity	heavy turbidity	heavy sediment
Deep agar	broken by gas col.lenticular	broken by gas	broken by gas col.lenticular	broken by gas col.lenticular
Growth in		turbid gas		
pep tone broth	turbid gas	sediment	turbid gas	turbid gas
Indole	slight /	slight /	slight /	رجا
•	slight	slight	slight	slight
peptone water	Tioccular	Tloccular	sediment	Tloccular
Ammonia	1		- 1	- 1
Milk Toaffler Ess mest	no digestion	no digestion	no digestion	no disestion
sult	4	+	•	1
Neutral red	changed	changed	changed	changed
dextrose broth	H	to ye	to yellow	to yellow
(0)	turbid sediment	turbid sediment	turbid	turbid
Nitrate reduction	. 1	- 1		¥
Glucose	- 1	1		slight acid
Galactose		light	- 1	
Sucrose	•	ight		ight
Mannitol	slight acid	- 1		slight acid
Maltose	Slight acid	slight acid	slight acid	slight acid
Inulin	acid	slight acid	slight acid	slight acid

(b. 3)
cocci
negative
Gram
Q

	L. 101	L. 115 A	L. 115 B	0 41 A
Agar slope	pin point	pin point	pin point	pin point
colonies	-11	coronriess	COTOUT + 620+101	foet.id
Odour	slight foetid	roetia	STIBILL TOGOTA	31000
Hemolysis	ı	1	1	1
ID	8	1	1	1
	1	1	1	1
gelatine	1	1	1	1
Liqueraction Serum broth	heavy turbidity	heavy turbidity	heavy turbidity	heavy turbid1W sediment
Deep agar	no visible gas	broken by gas	broken by gas col.lenticular	
ŀ ~┪	turbid	turbid gas	turbid gas sediment	turbld gas
peptone proun	J	7	+	slight /
144		slight	slight	slight flocular
peptone water	Troccular	LLOGGLAI	1 1 1	1
Ammonia	abanada on	no change	no change	no change
MIIK Icoffler Rog meat	177	1		no digestion
בקק <u>ה</u> לונש		-	7	7
Neutral red	changed	changed	changed to vellow	cnanged to vellow
1	to orange	turbid sediment	turbid sediment	turbidity
Ø]	STEMIN SOUTH	1	+	f
Nitrate reduction	المرد م م	slight acid	slight acid	slight acid
Glucose	1	1 1	slight acid	1
G:: Book	3	slight acid	1	- 1
Sucrose	•			
We 1+0se		slight acid		- [
Thullin	acid	slight acid	slight acid	slight acid

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4
අ)
cocci
negative
Gram
6

b) Gram negative co	cocci (p. 4)			
	Z. 24 B	AA. 401	DD. 3 A	DD. 17 A
Agar slope colonies	pin point colourless	pin point colourless	pin point colourless	small colourless
Odour	foetid	slight foetid	foetid	foetid
Hemolysis human blood	1	1	•	•
Hemolysis sheep blood	1	1	1	•
Hemolysis rabbit blood	1	ŧ	1	1
Gelatine	1	1	1	7
Serum broth	heavy turbidity sediment	heavy turbidity	heavy turbidity	heavy turbidity
Deep agar	broken by gas	broken by gas	! 1	broken by gas
Growth in	turbid gas	turbid, slight	turbid gas	turbid gas
ന	sediment	gas, sediment	sediment	sediment
1	slight /	${f slight} eta$	slight +	Silgnt 7
	• •	slight	slight godiment	slight sediment
peptone water	sediment	slight 4		1
Ammonia		obange ou	no change	very slight acid
Milk Toeffler Egg meat		177	no digestion	ŀ~ l
sult		1 1	+	
154	changed	changed	peguado	peguado
0	to yellow	to yellow	to yellow	to orange
Dextrose broth	sediment	sediment	turbid, sediment	sediment
ı H	<i>f</i>	+		
ł	•	1		- 1
Galac tose	slight acid		slight acid	- 1
Sucrose	ight	•	slight acid	- 1
Mannitol	1 1	•	- 1	8C1
Maltose	slight acid			1gnt
Inulin	slight acid	1	slight acid	slight acid

b) Gram negative cocci (p. 5)

	DD• 58	II.37 A&B	IL. 18 A	LL. 50 A
Agar slope colonies	pin point colourless	pin point colourless	pin point colourless	pin point colourless
Odour	slight foetid	slight foetid	foetid	foetid
Hemolysis human blood	1	1	1	1
Hemolysis sheep blood	1	1	1	1
Hemolysis rabbit blood	1	1	1 .	1
Gelatine liquefaction	1	1	1	1
Serum broth	heavy turbidity	heavy turbidity	heavy turbidity sediment	heavy turbidity sediment
Deep agar	broken by gas	broken by gas	broken by gas	broken by gas
i q	turbid gas	g 8	turbid gas	turbid gas
peptone broth	sediment	sediment	sediment	sediment
	$slight \neq$	$slight \neq$	slight \neq	slight ≠
7	# C C C C C C C C C C C C C C C C C C C	slight	slight	slight
peptone water	ALIBIT DAS	on an ines	allellthes	negrmens
Ammonita	1		- 1	
Loeffler Egg meat	no digestion	no digestion	no digestion	no digestion
sulp	1	Ź		7
151	changed	changed		changed
dextrose broth	— 1		НI	to yellow
Dextrose broth	slight turbidity	slight floccular	slight sediment	slight sediment
Nitrate reduction	7	•		- F
Glucose		Light	light	•
Galactose	slight acid	1	1	•
Sucrose		- 1	slight acid	
Mannitol	1		1	
Maltose		I		
Inulin	slight acid	slight acid	slight acid	1
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	K. 611	Q. 63	Y• 9 A	BB. 311
Gelatine	not liquefied	not liquefied	not liquefied	not liquefied
Veillon's Medium	tiny, lenticular	\triangleright	tiny, lenticular	tiny, lenticular
	colonies no visible gas	colonies no visible gas	colonies	colonies
Martin broth	turbid,	turbid	turbid	slight turbidity
	H2S slightly / slight odour		H2S slightly /	
Peptone water		very slight	very slight	
	growth, Hgs-	growth, H2S-	growth HgS-	
Meat broth	d	1	turbid	slightly turbid
	foetid odour		foetid odour	
Milk	no change	no change	no change	no change
Serum broth	turbid	slightly turbid	turbid	slightly turbid
Neutral red broth	no change	no change	no change	no change
Deep agar	tiny, lenticular	tiny, lenticular	tiny, lenticular	tiny, lenticular
	colonies	colonies	colonies	colonies
Deep blood agar	no change	no change	no change	
Peptone broth	slightly turbid	slightly turbid	slight sediment	
Egg white	œ١	no change	no change	no change
Blood broth	slight blackening HgS /	no change $slight\ HgS\ {m f}$	slight blackening slight H2S \neq	
Fresh organs	partial digestion H2S /	partial digestion H2S \neq		
Waltose	slight acid	3	acid	
Manni tol	1 1		3	
Dextrose	slight acid		acid	
Galactose	slight acid	slight acid	1	
Sucrose		1	acid	
Arabinose	slight acid	slight acid	acid	
Dulcitol	1			
Odour	foetid	foetid	not foetid	

d.1) Gram negative bacilli

a.1) Gram me							
	K.59A	K.102	K.130	L.74A	L.75	L.762	
Growth in broth	turbid	slight turb.	slight turb.		slight turb.	heavy sed.	turbid
Aerobic	-	-		-	-	-	-
Motility	-	-	-	-	-	-	-
Spore test	-		-	-	-	-	-
Gelatin		em	-	-	-	-	-
Milk	-	-	-	-	••	A	$\overset{\mathtt{A}}{\mathtt{clot}}$
Growth 250	<i></i>	7	+	<i>‡</i>	+	+	+
Growth 450	-	-	-	-	-	-	-
600 5 mins	. /	-	-	+	-	+	+
60° 10 mins	. /	-		/	-	+	/
60° 20 mins	• /	-	•	-	-	-	-
60° 30 mins		-	-	-	-	-	
Catalase	-	-	-	-	-	-	-
Indole	+	+	-	+	<i>f</i>	+	<i>f</i>
H ₂ S		7	+	<i>f</i> .	<i>f</i>	+	-
Nitrate reduction	-	-		-	-	-	-
Acetyl-meth Carbonil	yl _	-	-	-	-	-	+
Glucose	+	sl./	-	sl./	-	+	
Rhamnose	+	-	-	-		+	+
Mannitol	-	-	-	-	-	+	-
Trehalose	-	-	-	-	-	-	-
Arabinose	+	sl./	sl./	sl./	-	+	7
Xylose	7	sl./	-		-	+	<i>f</i>
Lactose	sl./	-	-		-	+	<i>†</i>
Sucrose	sl./	sl./	-	.	-	+	+
Maltose	+	sl.≠	-	-	-	7	+
Salicin	+	-	-	-	-	-	+

d.l) Gram negative bacilli (p. 2)

•			(F)				
	L.1022	0.35	0.37	0.39	0.72	0.90	Q.1
Growth in broth	turbid	turbid	slight turb.	slight turb.	turbid	heavy sed.	heavy sed.
Aerobic	-		-	-	+	<i>f</i>	-
Motility	••	-	-	-		•••	-
Spore test	-	-	-	-	-		-
Gelatin	-	-	-	-	-	•••	+
Milk	-	A		-		A clot	$^{\tt A}_{\tt clot}$
Growth 250	+	+	+	+	+	7	7
Growth 450	-	-	-	-	-	-	-
60° 5 mins	. /	-		-	+	+	+
60° 10 mins	. /	_	-	-	-	7	+
60° 20 mins	• -	-	-	-	-		7
60° 30 mins	• -	-	_	-	-	-	-
Catalase	-	+	-	+	-	-	-
Indole	<i>f</i>	+	+	+	+	-	+
H ₂ S	-	-	-	+	+	-	-
Nitrate reduction	-	-	-	-	-	-	-
Acetyl-meth	yl /		-	-	-	-	-
Glucose	+	+	4 00	-	+	7	+
Rhamnose	+	sl./		-		s1./	-
Mannitol	••	-	-	-	-	-	-
Trehalose	-	-	-	***	-	-	-
Arabinose	+	7	-	s1./	+	+	-
Xylose	+	+	sl./	sl./	-	+	-
Lactose	s1./	-	-	-	-	+	+
Sucrose	+	+	sl./	-	+	+	7
Maltose	+	-	.	-	+	+	7
Salicin	s1./	-	-	-	-	-	-

d.1) Gram negative bacilli (p. 3)

d.1) Gram ne	Racive	Dagiti	(p. a)				
	Q.2	Q.13	R.11	T.107	U.110	U.110 ₁	U,126
Growth in broth	heavy sed.	slight turb.	slight turb.	slight turb.		slight turb.	
Aerobic	-	7	-	-	-	-	-
Motility	-	-	-	-		-	-
Spore test	-	-	-	-	-	-	-
Gelatin	7	-	-	-	+	+	-
Milk	-	-	A	-	A	$^{\tt A}_{\tt clot}$	-
Growth 250	+	-	+	+	_	7	+
Growth 450	+	-	-	-	-	-	-
60° 5 mins.	. +	-	-	-	7	+	+
60° 10 mins.		-	-	-		-	-
60° 20 mins.		-	-	-	-	-	-
60° 30 mins		-	-	-	-	-	-
Catalase	+	-	-	-	-	-	+
Indole	+	7		-	-	-	+
H ₂ S	-	7	7	+	+	-	7
Nitrate reduction	-	-	-	-	-	-	-
Acetyl-methy Carbonil	yl /	-	-	-	-	-	-
Glucose	+	sl./	sl./	-	-	+	-
Rhamnose	sl.≠	-	-	-	-	-	-
Mannitol	+	-	-	-	-	-	-
Trehalose	sl./	-	-		-	-	-
Arabinose	-	+	sl./	-	-	+	sl./
Xylose	-	7	-	-	-	7	sl./
Lactose	-	-	-	-	-	7	-
Sucrose	-	sl./	-	sl.≠	-	+	-
Maltose	-	-	-	-	-	+	sl.≠
Salicin	-	-	-	-	-	-	-

d.1) Gram negative bacilli (p. 4)

d.1) Gran neg	Sautve	Dacill	T (D. 4)				
τ	J.129	W.35	X.61	X.79 ₁	DD.7	LL.25B	LL.76B
Growth in h	neavy sed.	heavy sed.	slight turb.	slight turb.			slight turb.
Aerobic	-	-	-	-	-	-	-
Motility	-			-	-	-	-
Spore test	-	-	-	-	-	-	-
Gelatin	+	-	-	-	-	-	-
Milk	-	$_{\tt clot}^{\tt A}$	-	-	-	-	<u>-</u>
Growth 250	-	<i>f</i>	7	+	+	-	7
Growth 450	-	+	-	-	-	-	-
60° 5 mins.	+	+	+	+	. <i>+</i>	+	+
60° 10 mins.	-	+		<i>f</i>	_	-	+
60° 20 mins.	-	7	en.	-	-	-	-
60° 30 mins.	-	-	-	-	-	-	-
Catalase	-	-		+	-	-	-
Indole	7	+	-	+	+	-	+
H ₂ S	-	+	7	-	+	7	+
Nitrate reduction	-	-	+	-	-	-	-
Acetyl-methy Carbonil	1 _	-	-	-	-	-	-
Glucose	+	+	-	-	7	-	7
Rhamnose	-	7	-	-	sl./	-	7
Mannitol	•••	-	-	-	sl.≠	-	-
Trehalose	-	sl./	-	-	7		sl.≠
Arabinose	•••	-	-	sl./	7	-	
Xylose	-	· /	-	sl./	sl.≠	-	7
Lactose	-	7	-	-	sl./	-	sl./
Sucrose	-	7	-	-	sl./	-	sl./
Maltose	-	7	-	-	-		-

d.2) Gram positive bacilli

	F.54B1	F.622	F.623	F.84A	G.911A	G.91 ₂ A	G.99A
Growth in broth	slight turb.	heavy sed.			heavy sed.	_	turbid
Aerobic	+	-	-	-	-	-	-
Motility	-	-	-	+	7	-	-
Spore test		-	-	-	-	-	-
Gelatin	+	· /	+	+	-	-	+
Milk	-	-	-	-	$^{\tt A}_{\tt clot}$	-	-
Growth 250	+	7	7	+	7	-	+
Growth 450		-	+	-	-	-	-
60° 5 mins.	· /	+	+	7	+	+	+
60° 10 mins.	· <i>†</i>	-	-	-	+	7	
60° 20 mins.		-	-	-	-	+	-
60° 30 mins.		-	-	-	-	-	-
Catalase	+	+	+	7	-	-	+
Indole	+	+	+	7	+	+	7
H ₂ S	+	-	-	-	-	-	-
Nitrate reduction	-	-	+		-	-	•
Acetyl-meth;	yl -	-	-	-	-	-	-
Carbonil Glucose	-	+	+	7	7	+	+
Rhamnose		-	-	-	-	-	-
Mannitol	-	+	7	+		-	-
Trehalose		-	sl./	-	sl./	-	-
Arabinose	-	-	-		+	sl./	· -
Xylose	-	-	-	-	+	+	-
Lactose	-	-	-	-	+	-	-
Sucrose	-	-	-	-	7	-	-
Maltose	•	-	-	-	+	sl./	-
Salicin	-	-	-	-	+	-	-

d.2) Gram positive bacilli (p. 2)

	G.99B	J.4A ₁	J.4C1	J.4D2	J.62	K.37	K.501
Growth in broth	slight turb.	turbid	heavy sed.	stringy sed.	turbid	heavy sed.	
Aerobic	-	+	-	7	-	-	+
Motility	-	-	-	-	-	-	-
Spore test	-	-	•	-	-	-	-
Gelatin	-	+	+	+	/	<i>f</i>	-
Milk	-	-	A	-	-	•••	$^{\tt A}_{\tt clot}$
Growth 250	-	+	+	<i></i>	+	+	/
Growth 450	-	-	-	-	-	-	-
60° 5 mins	• -	+	+	+	+	7	+
60° 10 mins		-	-	-	-	-	+
60° 20 mins		-	-	-	-	-	-
60° 30 mins	• -	-	-	-	-	-	-
Catalase	-	-	7	-	+	7	-
Indole	7	+	7	+	+	7	+
H ₂ S		-	-	-	-	-	-
Nitrate reduction	-	-	•••	-	-	-	-
Acetyl-meth	yl _	-	+	***	-	-	-
Glucose	sl./	sl./	7	sl.≠	+	+	
Rhamnose	-		-	-	-	-	-
Mannitol		-	-	-	-	7	-
Trehalose	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	sl./
Xylose	+	sl./	-	-	-	-	sl./
Lactose	-	-	-	-	-	-	-
Sucrose	-	-	•	-	_	-	
Maltose	-	-		-	sl./	-	-
Salicin	-	-	-	-	-	-	-

d.2) Gram positive bacilli (p. 3)

u.b) Gram p		<u> </u>					
	K.502	K.119 ₁	0.3	0.60	0.71	0.91	0.95
Growth in broth	slight turb.		slight turb.	turbid	turbid	slight turb.	slight turb
Aerobic	+	-	-	+	<i>\(\neq \)</i>	-	7
Motility		-	-	7	-	-	-
Spore test	-	-	-	-	-	-	-
Gelatin	-	•••	-	7	-	-	-
Milk	-	-	-	-	A clot	-	-
Growth 250	+	+	+	7	7	-	+
Growth 450	-	-	-	+	+	-	7
60° 5 mins	. /	+	+	+	7	-	+
60° 10 mins.	-		+	7	7	-	+
60° 20 mins		_	-	-	7	-	+
60° 30 mins			-	-	••	-	+
Catalase	-		-	+	+	-	-
Indole	<i>f</i>	-	+	<i>f</i>	+	7	+
H ₂ S	-	+	-		-	+	-
Nitrate reduction	+	••	-	-	-	-	.
Acetyl-meth	yl _	••	-	-	-	-	-
Glucose	1	sl./	+	+	-	-	7
Rhamnose	-	-	-	-	-	-	-
Mannitol	sl./	-	-	+	-	-	7
Trehalose	-	-	-	-	-	-	-
Arabinose	+		sl.≠	-	-	sl.≠	7
Xylose	+	7	+	-	-	sl.≠	+
Lactose	-	-	+	-	-	-	-
Sucrose	7	-	+	-	+	-	7
Maltose	sl./	-	-	-	+		7
Salicin	+	-	-	-	-	-	-

	0.95B	0.100	0.101	0.105	Q.7	Q.20	Q.27	L.109
Growth in broth	slight turb.	slight turb.	slight turb.		heavy sed.			turbi
Aerobic	-	+	+	+	-	-	+	-
Motility	-	-	-	-	-	-	-	+
Spore test	-	-	-	-	-	-	-	-
Gelatin	-	-	-	-	+	-		-
Milk	-	A	-	-	- '	-	-	A clot
Growth 250	+	+	7	-	+	+	7	<i>f</i>
Growth 450		+	+	-	-	-		
60° 5 mins	. /	-	-	-	+	+	+	7
60° 10 mins	. /	-	-	••		-	-	+
60° 20 mins	. /	-	-	-	-	-	-	+
60° 30 mins		-	-	-	-	-	•	-
Catalase	+	7	-	-	+	+	+	+
Indole	-	·/	+	-	-	+	+	7
H ₂ S	-	-	will	-	***	-	7	-
Nitrate reduction	+		-	-		-	-	-
Acetyl-methy Carbonil	yl _		-	-	-	-	-	-
Glucose	+	+	+	-	+	7	+	+
Rhamnose	-	sl./		-	-	+	-	
Mannitol	/	sl./	-	-	sl./	-	sl./	-
Trehalose	+	-		-	-		-	-
Arabinose	+	7	+	7	-	sl./	-	-
Xylose	+	+	+	-		-	7	-
Lactose	-	sl./	-	-	***	sl./	-	+
Sucrose	+	+	+	-	-	7	-	7
Maltose	-	+	+	sl./	-	-	-	7
Salicin	+	-	sl./	sl./	-	sl./	-	-

		2007777	(p. 0)				
	Q.27 ₂ A	Q.29	S.761	S.76	2 T.46	T.111	U.1071
Growth in broth	slight turb.		stringy sed.	heavy sed.	stringy sed.		slight turb.
Aerobic	-	-	-	-	-	7	-
Motility	<i></i>	-	+	+	7	-	-
Spore test	-	-	-	-	-	-	-
Gelatin	<i></i>	-	7	7	7	-	7
Milk	$_{\tt clot}^{\tt A}$	-	-	-	-	-	-
Growth 250	7	-	7	+	+	+	7
Growth 450	/	-	_	-	-	+	7
60° 5 mins	. /	-	7	+	+	7	-
60° 10 mins	. /	-	+	7	+	7	-
60° 20 mins		-	<i>f</i>	7	-	7	-
60° 30 mins		-	+	7	-	7	-
Catalase	-	-	-	-	-	-	-
Indole	<i>f</i>	<i></i>	-	-	-	+	-
H ₂ S	-	7	7	7	7	-	7
Nitrate reduction	-	-	-	-	-	-	-
Acetyl-meth	yl /	-	-	-	-	-	7
Glucose	-	sl./	-	-	-	+	-
Rhamnose	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-
Trehalose	-	-	-	-	-	-	-
Arabinose	-	+	-	-	-	+	-
Xylose	-	sl./	-	-	sl./	/	-
Lactose	-	-	-	-	-	+	7
Sucrose	7	sl./	-	-	-	7	-
Maltose	-	-	-	-	-	+	-
Salicin	***	-	-	-	-	7	-

4.67 41411 1	9001010		(b. 0)				
	U.123 ₁	U.127 ₁	W.19	W.45	X.20	X.38A	X.42
Growth in broth	turbid	slight turb.	slight turb.		slight turb.	slight sed.	slight turb
Aerobic	-	-	-	-	-	-	-
Motility	-	-		-	-	-	-
Spore test	-	-	-	-	-	-	-
Gelatin		-	-	-	-	-	-
Milk	-	-	-	-	A	-	-
Growth 250	+	-	7	-	+	-	-
Growth 450	-	-	-	_	-	-	-
60° 5 mins	• +	+	+	-	+	+	+
60° 10 mins	. /	+	-	-	+	-	+
60° 20 mins		+	-	-	-	-	-
60° 30 mins		-	-	-	-		-
Catalase	-	-	_	-	••	-	-
Indole	+	+	-	7	7	+	+
H ₂ S	-	-	-	+	+	+	+
Nitrate reduction	-	-	7	-	-	-	+
Acetyl-meth	nyl _	-	-	-	-	-	
Glucose	+	+	-	sl./	•	-	7
Rhamnose	-	sl./	-	sl./		-	sl./
Mannitol	_	sl./	-	-	-	-	-
Trehalose	-	-	-	-	-	-	-
Arabinose	-	+	-	7	-	sl./	7
Xylose	sl./	+	+	-	-	+	+
Lactose	-	sl./	-	-	-	sl./	+
Sucrose	-	7		sl.≠	-	sl./	+
Maltose	/	+	-	-	-	-	sl./
Salicin	-	+	-	-	-	-	

				<u> </u>			
	X.71 ₁	Y.3	Y.17	EE.5	KK.29	KK.40	MM.36
Growth in broth	slight turb.	heavy sed.	Turb.	Turb.	slight turb.	slight turb.	heavy sed.
Aerobic		-	-	-	+	+	-
Motility	-	+	sl.≠	-	-	-	-
Spore test		-	-	-	-	-	-
Gelatin	-	+	+	-		-	+
Milk	-	A	-	$_{\tt clot}^{\tt A}$	A	-	-
Growth 250	+	clot /	7	4	+	-	+
Growth 450	-		-	+	-	-	-
600 5 mins.	<i>+</i>	<i>‡</i>	+	+	+	7	+
60° 10 mins.		-	+	+	-	_	+
60° 20 mins.	, -	-	-	7	-	-	-
600 30 mins.		-	-	-	-	-	-
Catalase	-	1	-	•••	+	-	+
Indole	-	+	+	+	+	7	+
H ₂ S	+	-	-	-	+	7	-
Nitrate reduction	-		••	-	-	-	-
Acetyl-methy Carbonil	yl _	+	7	7	-	•••	-
Glucose	+	7	+	+	+	+	+
Rhamnose	-		-	-	-	-	-
Mannitol	sl./	7	-	7		-	7
Trehalose	-	-		-	-	-	sl./
Arabinose	7	-	sl.≠	-	sl./	-	-
Xylose	-	-	-	-	sl./	sl.≠	-
Lactose	+	-	+	+	sl./	-	-
Sucrose	7	-	sl./	+	sl./	-	-
Maltose	-	-	-	+	-	-	-
Salicin			-	-	sl./	-	-

APPENDIX II FORMULAE OF MEDIA

The formulae for Difco products may be found in the Difco catalogue.

Beef-cysteine medium for indole test (58)

Bacto tryptone

2 gm.

Bacto beef extract

1 gm.

Cysteine hydrochloride

.05 gm.

Water

100 ml.

Calf brain broth (McGill University)

Beef heart infusion broth

100 ml.

Calf brain, chopped to 1 ml.size 30 gm.

Autoclaved 120°C. for 20 minutes.

Cooled

Filtered

pH adjusted to 7.8 with phenol and N/10 NaOH.

Refiltered

Tubed and sterilized

Egg white coagulated

Egg white

2 parts

Peptone broth

1 part

Beaten with sterile precautions

Tubed, sloped, inspissated in drying oven.

Glucose-cysteine broth (58)

Difco Bacto tryptone 20 gm.

Difco Bacto beef extract 10 gm.

Glucose 8 gm.

Cysteine hydrochloride 0.5 gm.

Disodium phosphate (hydrate) 4 gm.

Distilled water

Glucose-cysteine semisolid

Difco Bacto agar 0.35 gm.

Glucose-cysteine broth 100 ml.

Glucose-cysteine agar

Difco Bacto agar 2.0 gm.

Glucose-cysteine broth 100 ml.

Hiss serum (McGill University)

Sheep serum containing 0.2% formalin 1 part

Distilled water 3 parts

Adjust to pH 7.2 to phenol red with N. HCl.

Autoclave 120 C. for 20 minutes

Adjust pH 7.2

Reautoclave

Add proteose peptone 0.3%

Andradi indicator 1.0%

Adjust pH 7.2

Add dry sugar 1.0%

buffer solution 1.0%

Tube and autoclave

Loeffler's blood serum (McGill University)

Ox serum 3 parts

1% dextrose broth 1 part

Adjust pH 8.0 to phenol red with NaOH.

Tube, bake in autoclave in sloping position

Martin broth (McGill University)

Pigs' stomachs, uncleaned, prepared by removing fat and mincing.

Distilled water at 55°C. 1000 ml.

Concentrated HCl. 10 ml.

While steaming add minced stomach 300 gm.

Digest for 7 - 8 hours at 50°C.

Raise the temperature to 90°C. for 15 minutes.

Bottle

Filter, tube and sterilize for use.

Nitrate broth

Distilled water 1000 ml.

Proteose peptone 10 gm.

Potassium nitrate 2 gm.

Filter, tube and autoclave

Peptone agar (McGill University)

Minced beef heart, free of fat 500 gm.

Distilled water 1000 ml.

Heat at 75 - 80° C. for $1\frac{1}{2}$ hours.

Filter through paper pulp in a Buchner funnel under slight suction.

Melt agar shreds, washed and dried 1.5%

in 1/10 volume of meat infusion

Peptone agar (McGill University) (Cont'd)

Add proteose peptone

1%

NaCl

0.25%

KC1

0.02%

CaClo

0.01%

Add to bulk of meat infusion

Cool overnight

Melt at 100°C.

Adjust pH to 8.5

Heat at 120°C . for 20 minutes to precipitate phosphates.

Filter through paper pulp

Adjust pH 7.2 with HCl.

Sterilize at 120°C. for 20 minutes

Check pH.

Bottle and autoclave.

Peptone broth (McGill University)

Minced fresh beef heart, free from fat 1 lb.

Distilled water

1000 ml.

Heat at 75 - 80° C. for $1\frac{1}{2}$ hours.

Filter through paper pulp in Buchner funnel under slight suction.

Add proteose peptone

1%

NaCl

0.25%

KC1

0.02%

CaCla

0.01%

Adjust pH 8.5 to phenol red with N. HCl.

Heat at 120°C. for 20 minutes

Filter through paper pulp

Adjust pH 7.2

Bottle and autoclave.

Peptone water (McGill University)

Distilled water	
Difco proteose peptone	1%
NaCl	0.25%
KCl	0.02%
CaCl ₂	0.01%
Adjust pH 7.0 to phenol red with	NaOH
Heat at 100°C.	
Filter	
Adjust pH 7.0 to phenol red	
Bottle and autoclave	
Add dry sugar	0.5%
1.6% Brom cresol purple	0.1%
Tube and autoclave	
Potato extract medium (100)	_
Proteose peptone	1.0%
Difco beef extract	0.3%
Cysteine hydrochloride	0.05%
Glucose	0.1%
Potato extract	0.0%
Bacto agar	2.0%
Distilled water	
Adjust pH 7.6	

Gentian violet

1:20,000

Potato extract (95)

Grind deeply peeled potatoes

Let stand ground potato

100 gm.

Distilled water

100 ml.

in ice box for 24 hours

Filter through cheese cloth

Filter through filter paper under suction

Boil 2 - 3 minutes to complete coagulation

Filter through paper under suction.

Serum (pneumo broth) (McGill University)

Fresh minced beef heart, free of fat 500 gm.

Distilled water

1000 ml.

Heat at 75 - 80° C. for $1\frac{1}{2}$ hours

Filter through paper pulp in Buchner funnel under slight suction

Add proteose peptone

2%

Adjust pH 7.2 to phenol red with N/10 NaOH

Heat at 120°C. for 10 minutes

Cool

Filter through paper

Adjust pH 8

Heat at 120°C. for 10 - 15 minutes

Filter

Add dextrose

0.1%

Bottle and autoclave

Before distributing add

K2HPO4

0.2%

sterile rabbit serum

5.0%

Tube sterilly

Serum coagulated

Sterile human or rabbit serum

Tubed and inspissated in drying oven

Thioglycollate meat medium

Robertson's meat mash:

Grind lean beef

Cover with slightly basic water

Simmer 20 minutes

Cool, pour fat off

Wash to remove remaining fat

Partly dry in oven

Fill screw capped test tubes to one inch

Add 10 - 12 ml. Brewer thioglycollate medium

Autoclave at 120°C. for 20 minutes.

Thioglycollate medium:

Brewer modified of Baltimore Biological Laboratory

Peptone 20 gm.

Dextrose 10 gm.

NaCl 5 gm.

Disodium phosphate 2 gm.

Sodium thioglycollate 1 gm.

Methylene blue 0.002 gm.

Agar 0.5 gm.

For use, 35 gm. of the above formula melted in 1000 ml. distilled water.

Veillon's semisolid (114)

Stand 500 gm. chopped lean beef in 1000 ml. distilled water for 24 hours.

Filter through cheese cloth, squeezing meat Replenish liquid to 1000 ml.

Add peptone

10 gm.

NaCl

5 gm.

Agar

12 gm.

Heat in autoclave at 100°C. to melt peptone and salt, and precipitate the albumen

Make pH slightly alkaline

Heat at 120°C. for fifteen minutes in the autoclave Melt 15 gm. glucose in a little water and add Cool to 50°C. Add one egg white, well beaten.
Autoclave at 120°C. for 10 minutes.

Filter through paper

Tube, to a height of 10 cm. in tubes of 18 x 150 mm. Sterilize.

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