NON-SPOREFORMING ANAEROBIC BACTERIA OF MEDICAL IMPORTANCE

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The non-sporulating anaerobic bacteria, although discovered at about the turn of the century, are as little known to most bacteriologists today as they were thirty years ago. The textbooks of bacteriology give little if any discussion of them, so that it becomes a major research problem for the laboratory worker to identify any of these species when encountered. The importance of the non-sporulating anaerobic bacteria in medical bacteriology is controversial. At the Third International Congress for Microbiology in the section on Anaerobes the following statement was made by Thompson (1): “In clinical bacteriology, anaerobes play a minor role. The finding of anaerobes is analogous to the occurrence of red-letter days on the calendar—when they occur they are usually worthy of consideration.” With this statement in mind, inquiry was made regarding the incidence of non-sporulating anaerobes in specimens submitted for bacteriological examination in the Department of Surgery at the University of Chicago, Billings Hospital. From July, 1936 to May, 1940, the following results were obtained:

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>TOTAL NUMBER OF SPECIMENS EXAMINED</th>
<th>NUMBER OF SPECIMENS CONTAINING NON-SPORULATING ANAEROBES</th>
<th>PER CENT CONTAINING NON-SPORULATING ANAEROBES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus..............</td>
<td>2,621</td>
<td>146</td>
<td>5.57</td>
</tr>
<tr>
<td>Fluids............</td>
<td>786</td>
<td>18</td>
<td>2.29</td>
</tr>
<tr>
<td>Tissues...........</td>
<td>428</td>
<td>16</td>
<td>3.73</td>
</tr>
<tr>
<td>Gall bladder wall.</td>
<td>340</td>
<td>13</td>
<td>3.82</td>
</tr>
<tr>
<td>Bile..............</td>
<td>371</td>
<td>3</td>
<td>0.80</td>
</tr>
<tr>
<td>Blood.............</td>
<td>634</td>
<td>4</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5,180</strong></td>
<td><strong>200</strong></td>
<td><strong>3.86</strong></td>
</tr>
</tbody>
</table>

1 The author is indebted to Miss Irma Holicky, Bacteriologist, Department of Surgery, University of Chicago, for collecting these data.
The 200 specimens contained 230 non-sporulating anaerobes as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Gram-variable</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Streptococci</td>
<td>1</td>
<td>α 13, β 4, γ 45</td>
<td>5</td>
</tr>
<tr>
<td>Black colonies (Strep.)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cococbacilli</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Rods</td>
<td>6</td>
<td>9</td>
<td>46</td>
</tr>
<tr>
<td>Fusiforms</td>
<td>0</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>0</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Curved diphtheroids</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Of the 200 specimens containing non-sporulating anaerobes, 54 contained them in pure culture, 20 in mixture with other anaerobes, and the remaining 119 in mixture with both anaerobes and aerobes. It is obvious from the above figures that non-sporulating anaerobes were encountered in routine surgical bacteriology on the average of about 50 times a year and were present in approximately 4 per cent of the total specimens examined. This incidence is too high to be ignored in any routine cultural studies.

McCoy and McClung (2) in their bibliography of the anaerobes list some 266 species of non-sporulating anaerobes with a total of 831 references. Of the 266 species, however, only 16 have received much attention. Two of these may be omitted from this discussion, one, Bacteroides bifidus, is more properly grouped with the lactobacilli, and the other, Spirillum desulfuricans, is not of importance in medical bacteriology. The remaining 14 with which this review is concerned are as follows (the numbers in brackets following each organism represent the number of references listed for the species):

- *Staphylococcus parrulus* [45]
- *Streptococcus anaerobius* [17]
- *Streptococcus foetidus* [25]
- *Streptococcus putridus* [30]
- *Bacteroides fragilis* [63]
- *Bacteroides funduliformis* [72]
- *Bacteroides furcosus* [15]
- *Bacillus fusiformis* [151]
- *Bacterium melaninogenicum* [18]
- *Bacillus nebulosus* [15]
- *Bacterium necrophorum* [64]
- *Bacterium pneumosintes* [47]
- *Bacillus ramosus* [56]
- *Bacteroides serpens* [17]
Several generic names are cited for a single species, which is in keeping with the confusion now existing in bacteriological nomenclature. Recently Prévot (3) has proposed new generic names for the anaerobes. Bergey’s Manual (3a) classifies the gram-negative members of this group under the generic name of Bacteroides. Since these organisms are so different in morphology and physiology, it seems absurd to group them under one genus. In this review mention will be made of some of the generic and specific names applied to these microorganisms, but no attempt will be made to name them. As will be pointed out subsequently, we do not know enough about many of them to assign them to special genera.

Most of the work on the non-sporeforming anaerobes has been done by clinicians, who have isolated and described new species. The descriptions of many species have been so inadequate that the same organism may be again discovered by another investigator and given a new name. Another difficulty is that no two workers will use the same technique in isolation and identification, thus affording no comparative basis for the examination of newly isolated strains. In many hospitals no effort is made to look for this group of organisms, hence the laboratory diagnosis of “sterile pus” is made on specimens from abscesses, if no growth appears on aerobic blood-agar plates.

Nearly all the non-sporulating anaerobes of medical importance are normal inhabitants of the mucous membranes of the body, inhabiting the upper respiratory tract, the colon and the genital tract. Under conditions which give rise to necrosis of the mucous membranes one or more of these species may become established and invade the tissue. It is when they produce abscesses or enter the blood stream that they are usually detected. They are not found at their original portal of entry because of poor methods for their isolation. Most workers in bacteriology seem to think that a search for anaerobes is too arduous a task to attempt, although with modern apparatus it is possible to isolate and study anaerobes in relatively convenient routine fashion. In the author’s experience the early method proposed by Veillon for the isolation of anaerobes is entirely adequate when the
anaerobe sought is greatly outnumbered by other bacteria. No originality is claimed for the method used successfully in this laboratory for many years. The method is as follows: A blood-agar plate containing 10 per cent of sheep blood is streaked with specimens to be examined. When the specimen is heavily contaminated a second plate is streaked from the first. Anaerobic conditions are provided by using ordinary pyrex desiccators with ground glass stoppers. The ground joints of the jar are sealed with a preparation made of equal parts of rubber, paraffin, and vaseline. This sealing preparation is effective at incubator temperatures. The plates after streaking are placed in the jar over a solution of pyrogallic acid and sodium carbonate. The jar is closed and evacuated with an oil pump to approximately 10 cm. mercury pressure. Carbon dioxide is allowed to flow into the jar until atmospheric pressure is reached, after which the jar is again exhausted to 25 cm. mercury pressure and is then sealed and placed in the incubator. Oxygen dissolved in the blood agar is absorbed by the sodium pyrogallate. The blood-agar assumes a cyanotic color when good anaerobic conditions are attained, thus serving as an indicator of the relative absence of oxygen in the jar. Non-sporeforming anaerobes of diverse types have been successfully isolated by this simple procedure.

ANAEROBIC COCCI OF MEDICAL IMPORTANCE

Numerous species of anaerobic cocci have been described, but only four have been sufficiently studied to warrant space in this review.

_Staphylococcus parvulus_. This organism has also been listed under the genus _Micrococcus_, although Weinberg et al. (4) have placed it in a genus _Veillonella_, because it is gram-negative and differs from the members of the genus _Neisseria_ in having an interstitial substance (ectoplasm) which may be demonstrated by Giemsa's stain. This organism was first described by Veillon and Zuber (5), who claimed to have found it in pus in appendicitis, either in the abscesses about the cecum or in the peritoneal cavity in a generalized peritonitis. Subcutaneous abscesses have been produced in rabbits and guinea pigs with these strains.
These workers noted the predominance of this organism over *Escherichia coli*. All cultures produce gas and a very foul odor. Gelatin is not liquefied and milk is not affected. Glucose is fermented and certain strains apparently attack other carbohydrates (3, 4), although there seems to be considerable irregularity in this respect.

*Streptococcus anaerobius* and *Streptococcus foetidus*. These two species are listed together, since the description of their activity in different media is identical. The only difference ascribed to them is in morphology, and with the recognition of variation among other species of bacteria, it does not seem at the present time that this difference is in itself adequate to separate these as two distinct species. *Streptococcus anaerobius* is described as having long regular chains, whereas *Streptococcus foetidus* appears in short chains and irregular arrangement with occasional tetrads. *S. anaerobius* was first described by Krönig and Menge (6) and *S. foetidus* by Veillon (7).

Both organisms are gram-positive and are generally found in the oral cavity, intestine and vagina in abundance. They produce gas and foul odors in all media. Gelatin is not liquefied and neither milk nor heat-coagulated proteins are affected, although good growth occurs in serum and a very foul odor results. In man, the organisms have been found in purulent gangrenous processes involving the genital tract, lungs and viscera, and in septicemias. They do not produce strong toxins, neither do they cause hemolysis. There seems to be a difference of opinion among workers regarding their pathogenicity for animals. Prévot (3) claims that local edematous lesions with gangrenous suppuration have been produced in guinea pigs with *S. foetidus*.

*Streptococcus putridus*. Schottmüller (8) first described *Streptococcus putridus* and reported 25 cases in which it was found in the lesions. These cases comprised meningitis, cystopyelitis, gangrene of the lung, salpingitis with pelvic abscesses, and septic abortion (ten cases, of which eight had thrombophlebitis with other complications). *S. putridus* was isolated in pure culture in 12 of the cases, and found along with other organisms in the rest.
Schottmüller observed that on artificial media long and short chains were present and the individual organisms were usually flattened and appeared attached as diplococci. In old cultures he noticed different shapes, with some cells appearing as rods. The strains were all gram-positive. S. putridus was often isolated from blood by placing the blood immediately in bouillon and incubating it without mixing or further handling. On blood-agar plates the colonies were porcelain white in color, and of the size of the head of a needle.

The main characteristic by which S. putridus may be differentiated from other anaerobic streptococci is the reaction in blood-broth cultures. The blood takes on a characteristic poppy-red color; and spectroscopically H₂S may be demonstrated in blood cultures. In about ten days blood-broth cultures are black in color. Schottmüller believed S. putridus was not a simple saprophyte, since it was able to invade and produce lesions elsewhere in the body. He called attention to the ability of this organism to dissolve fibrin, since in the pleural exudates one never finds the slightest trace of fibrin clots.

S. putridus is strictly anaerobic. Heat-coagulated protein media are not attacked, whereas sterile protein solutions such as serum are attacked and give rise to a foul odor. Glucose, levulose and maltose are fermented (3).

Since Schottmüller's original description of S. putridus in 1910 (8) there has been little attention given this organism. A few reports have appeared, some of which will be mentioned. Schwarz and Dieckmann (9, 10) in St. Louis made a search for this organism in puerperal infections. Of 165 uterine cultures and blood cultures from suspected cases, they found that 46 contained S. putridus (9). More recently Stone (11) has studied 26 strains of anaerobic streptococci, which he isolated from parturient and post-abortal women by means of a sterile pipette inserted through the cervix into the uterus. The organisms were found to be gram-variable. Stone attempted to apply some of the tests used in differentiating aerobic streptococci of the beta-hemolytic type, such as growth in 10 and 40 per cent bile, final pH in glucose medium, hydrolysis of sodium
hippurate, fermentation of trehalose and sorbitol, and finally the precipitin reactions. He found that it was impossible to set up definite groups of these strains by means of their precipitin reactions. Stone made no attempt to differentiate S. putridus from other anaerobic streptococci by its reaction in blood-broth, as recommended by Schottmüller (8).

**ANAEROBIC NON-SPORULATING GRAM-NEGATIVE RODS**

Organisms of this group have been placed in many genera. Castellani and Chalmers (12) have listed a Tribe Bacteroideae in the Family *Bacillaceae* with the following description: “Bacillaceae with good growth on ordinary laboratory media, without endospores, fluorescence, or pigment formation, and obligatory anaerobes.” They named a genus *Bacteroides* with these tribal characters and *Bacteroides fragilis* was given as the type species. This generic name, *Bacteroides*, includes both gram-negative and gram-positive species, although it is proposed (13) that it be restricted to the gram-negative species.

From the author's experience with the gram-negative non-sporulating organisms, it seems unwise to place all of these in a single genus, since they represent greatly different morphological types. In this review, however, these organisms will be listed under the names appearing in the McCoy and McClung bibliography (2).

*Bacteroides fragilis*

Prévot (3) has listed this organism in a genus which he calls *Ristella*, and defines as containing asporulating simple rods, non-ciliated, non-motile, straight or slightly curved, non-capsulated and gram-negative. Under this genus he has listed 25 species. Topley and Wilson (14) list this organism in a genus *Fusiformis*, which they characterize as “obligate parasites, anaerobic or microaerophilic. Cells frequently elongated and fusiform, staining somewhat unevenly. Filaments sometimes formed; non-branching. Non-motile. No spores. Reaction to Gram variable. Growth in laboratory media feeble.” Veillon and Zuber (5) called this organism *Bacillus fragilis*. In addition
to having four different generic names, Bacillus, Bacteroides, Fusiformis and Ristella, it has another species name: sassmannshausen, given it by Heyde in 1911 (4).

The description given this organism by Veillon and Zuber (5), who found it in 22 cases of appendicitis, may be translated as follows:

"This bacillus appears to us to be the most abundant and the most constant in the pus from appendicitis. It is a fine rod, a little smaller than that of diphtheria, rounded at the ends and regular. It presents itself in the form of rods, isolated or united two by two by one of their extremities. Sometimes certain bacilli are slightly curved. In culture they have the same appearance, although they appear a little larger and certain rods are longer. It is gram-negative and non-motile. Although this bacillus is in great abundance in the pus from appendicitis, it is difficult to isolate. At incubator temperature the colonies do not appear in the depth of the agar until the third or fourth day. They form little round or slightly irregular colonies, ovoid, brownish yellow, rather opaque with smooth borders. These colonies remain discrete and although they are not far separate, one from the other, they remain punctiform. The most isolated colonies are less than one millimeter in diameter, and it is necessary to transplant them as soon as they are evident because they die quickly. A culture left 7 or 8 days in the incubator is no longer viable. On agar at the surface this organism forms extremely fine colonies, very transparent, grayish, scarcely more marked than those of pneumococcus and, like those, at the end of several days they become less visible and seem to be reabsorbed.

"Cultures can be obtained on gelatin at room temperature. The colonies which appear at the end of 8–10 days are punctiform, yellowish granules with wet edges. The medium is not liquefied. The culture is viable for 20–30 days. In broth growth occurs easily and in relative abundance. The medium is uniformly cloudy and there is a fine whitish deposit at the bottom of the receptacle. The cultures do not give off enough gas to break up the agar, but do give off a fetid odor. We have not established the production of spores and, as we have said, this bacillus is very fragile and non-resistant. It is pathogenic for guinea pigs and forms abscesses when injected subcutaneously. If the animal does not die of its abscess, the pus is eliminated and the guinea pig becomes cachectic and dies in about a month. This bacillus is much more virulent for rabbits. By subcutaneous inoculation there is produced a
large phlegmon with separation of the skin and death in 6 to 7 days. An inoculation in the veins produces death by cachexia; but one is unable to find the bacilli in the body. It is probable that toxins are the agents in this case, because one obtains the same results with dead cultures.”

This description given by Veillon and Zuber is useful in the isolation and identification of the organism. Cohen (15) listed this organism in 4 out of 16 cases of abscess of the lung, although his summary stated that he found it five times. His strains fermented maltose, glucose, sucrose and lactose, but not mannitol and inulin. Litmus milk was not acidified and gelatin not liquefied. Henthorne, Thompson and Beaver (16) isolated strains from the following: pelvic abscess in a patient with carcinoma of rectum, hepatic abscess and appendix in a patient with gangrenous appendicitis, abscess over sacrum in a patient with pilonidal cyst, and from the appendix in gangrenous appendicitis. Three of these cases were fatal. Their strains fermented glucose, maltose, lactose, sucrose, levulose, inulin, dextrin, xylose, raffinose, galactose and glycogen. Only one of the 4 strains fermented rhamnose, arabinose and trehalose, whereas none of them fermented mannitol, inositol, dulcitol, glycerol, salicin or sorbitol. They did not produce H₂S, hemolysis on blood-agar plates, reduction of nitrates or liquefaction of gelatin. Acid coagulation was produced in milk and gas was produced in the carbohydrates that were fermented.

Bacteroides fragilis is not limited to lesions about the appendix or the intestinal tract. It has been found, as previously mentioned, in lung abscesses as well as in many other conditions, such as periurethral and other infections of the urinary tract. It has also been found in septicemias with metastatic abscesses. No toxins have been demonstrated, in spite of the suggestion of their presence by Veillon and Zuber.

Not all workers are in agreement with Veillon and Zuber regarding the pathogenicity of these strains for rabbits and guinea pigs. The problem of differences of opinion concerning pathogenicity will be discussed in the review under Bacterium necrophorum.
Bacteroides funduliformis and Bacterium necrophorum

These two organisms are grouped together, since they have common properties and there seems to be no good reason for classifying them separately. Damman (17) in 1884 probably saw Bacterium necrophorum in the lesions of calf diphtheria. Loeffler (18) in the same year observed the organisms in calf diphtheria and succeeded in producing necrotic lesions in mice by subcutaneous inoculation of the necrotic membrane. He obtained a primary isolation of the organism from mice on calf serum but failed to subculture it. In 1891 Schmorl (19) reported an epidemic among rabbits in his laboratory, which was characterized by caseous necrotic lesions of the mucosa. He isolated an organism from the lesions which he named Streptothrix cuniculi. Much work by veterinary bacteriologists followed these early studies and consequently infections caused by Bacterium necrophorum are commonly recognized.

Bacteroides funduliformis may have been first studied by Veillon and Zuber in 1894 (5) and described by them as species C. The first clear-cut recognition of this organism was by Hallé (20), who found it in the vagina in the healthy state, in exudates from retained placentas, and in pus in Bartholinis. He described the organism in pus as a rod, generally slightly curved. He observed that when it is the only organism in pus it is never very abundant, that it does not stain well and that sometimes its ends are better colored than its center.

Bacterium necrophorum has received numerous names, which follow (3, 4):

Bacillus of Schmorl (Weinberg et al.)
Bacillus necrophorus (Flügge)
Actinomyces necrophorus (Bergey, 1930)
Bacillus necrosus (Jensen)
Bacillus diphtheriae vitulorum (Flügge)
Bacillus filiformis (Schütz)
Nekrosebacillus (Bang)
Streptothrix cuniculi (Schmorl)
Actinomyces cuniculi (Gasperini)
Bacillus necroseos (Salomonsen)
Bacillus des Kälbernoma (Ritter)
Streptothrix necrophora (Kitt)
NON-SPOREFORMING ANAEROBIC BACTERIA

Actinomyces necrophorus (Neukirch)
Corynebacterium necrophorum (Lehmann and Neumann)
Fusiformis necrophorus (Topley and Wilson)
Corynebacterium de la necrose (Hornach)
Spherophorus necrophorus

The names given Bacteroides funduliformis (4) are:

Espèce C (Veillon and Zuber, 1894)
Bacterium funduliforme
Bacillus funduliformis
Bacillus thetoides (Rist and Guillemot, 1898)
Spherophorus funduliformis

This multiplicity of names is sufficient evidence for the confusion in the isolation and identification of these organisms. Prévot (3) has recently given the family name SPHEROPHORACEAE to the gram-negative organisms in the Class ACTINOMYCETALES. He has given the generic name Spherophorus and defined it as follows: Rods, straight or slightly curved, very polymorphic, occurring in exudates, ovoid with bipolar staining, in cultures forms are variable: filamentous, swollen, in form of sausage, ramified with constant presence of spheroids of variable shape, sometimes very large, metachromatism in elongated and filamentous forms, non-motile, non-ciliated, non-sporeforming and gram-negative.

This generic description by Prévot is sufficient excuse for the many names which these organisms have received. Animal and human strains of Bacterium necrophorum grow well on the surface of anaerobic blood-agar plates, prepared according to the method previously described. When the plates are removed from the anaerobic environment and exposed to air, a greenish zone appears about the colonies, which upon prolonged exposure to the air may change to a clear hemolysis. In the anaerobic state when the hemoglobin is reduced no hemolysis may be seen about the colonies. Colonies vary in size on different anaerobic blood-agar plates; sometimes they appear very small and at other times large. No reason can be ascribed for this condition. There is no significant difference in the colonies from human and animal origin (21). A foul odor is produced in all cultures.
The morphology of the cells of *Bact. necrophorum* is variable, as Prévot (3) has stated in the definition of the genus in which he places these organisms. The morphology varies with the type of medium used, so that it may even be questioned whether cultures are pure (Hallé, 20). In general, animal strains produce long, filamentous forms in broth and in anaerobic blood-agar slant cultures, whereas human strains have more "ghost forms" and short forms. Irregular staining and granules are commonly found in the cells. The morphological difference, however, is not absolute or clear-cut enough to warrant making species differentiation. All strains are gram-negative.

**Biochemical reactions.** Many difficulties are encountered in determining the biochemical properties of this group of bacteria. Some strains fail to grow in a basic medium of veal infusion broth or they grow with great irregularity, unless a fermentable carbohydrate is present. The addition of 10 per cent serum, 0.05 per cent cystine or 0.1 per cent cysteine has been found effective (21) in supporting growth in the basic medium. Glucose, maltose and levulose are fermented and, in general, more acid is produced from glucose and levulose than from maltose. Lactose, sucrose, mannitol and glycerol are not fermented and, litmus milk is unchanged. Indole is produced in tryptophane veal infusion broth containing 0.05 per cent cysteine. Gelatin is not liquefied and none of the strains digests coagulated egg white. Many strains cause a drop in pH of only about 0.1 in the basic medium of veal infusion broth containing 0.05 per cent cysteine and frequently produce a small amount of gas in solid agar medium. The irregularity of growth due to the sensitivity of these organisms to oxygen, together with the property which some of them possess of producing slight amounts of acids and gas in basic medium, probably accounts for the lack of uniformity in the reported biochemical reactions.

**Pathogenicity for animals.** When strains isolated from many animal lesions are injected subcutaneously into the rabbit, a spreading necrotic lesion develops which kills the animal in 6 days or longer. Not all strains are lethal in this way. Orcutt (22) found that one of 10 cultures isolated from a bovine liver
abscess produced only a local abscess upon subcutaneous injection into a rabbit. Some of the human strains produce spreading lesions and death, but usually only local abscesses (23, 24) which are slow in healing. Organisms may be found in the pus for long periods of time (23). Intravenous injection of human strains sometimes gives rise to joint lesions.

In experimental infections in rabbits sulfanilamide has given good therapeutic results (25), although in certain infections in man the results have been discouraging (26).

The guinea pig appears to be quite resistant to human strains of Bacterium necrophorum (27). This is in contrast to the results of Hallé (20), who reported abscesses from subcutaneous injections. Guinea pigs on a vitamin C deficient diet, however, readily develop lesions when injected with human strains (27). It may well be that the success which early investigators had in producing lesions in laboratory animals was due to the deficient diets of their experimental animals.

**Immunological reactions.** The problem as to whether Bacterium necrophorum produces toxins is one that has been much debated. Beveridge (28) in a study of 12 animal strains concluded that these organisms produce a soluble toxin and an endotoxin, the latter being resistant to heat and chemical agents. He claimed to demonstrate exotoxins by filtering a broth culture and injecting rabbits intradermally with 0.1 ml. of filtrate. Subcutaneous inoculations of rabbits with 1 to 3 ml. of filtrate produced no obvious local reaction, but a slight hemorrhagic appearance to underlying muscles was observed through the skin. Four milliliters of a Berkefeld N filtrate given intravenously to a 2000-gram rabbit killed the animal in one hour, whereas 3 ml. of a “Seitz EK special” filtrate similarly given to a 1500-gram rabbit caused collapse in one hour, but the rabbit survived. In guinea pigs, 1.5 ml. of a fresh Chamberland L3 filtrate inoculated intravenously or subcutaneously had no effect, whereas 0.1 ml. intradermally produced only very slight swellings 0.5 cm. in diameter. One to 2 ml. of whole culture intravenously killed 3 of 4 guinea pigs in from 4 to 20 hours. A sheep inoculated intravenously with 20 ml. of fresh Berkefeld N filtrate
developed diarrhea, labored breathing and anorexia for 3 days, then recovered.

Beveridge (28) demonstrated endotoxins by treating suspensions of the organisms in different ways, such as (a) formalinizing (0.5 per cent) and incubating for one and for 8 weeks at 37°C., (b) heating at 100°C. for one hour, and (c) heating at 60°C. for 15 minutes. All of these preparations, when inoculated intradermally into rabbits in doses of 0.1 ml., produced well-marked nodular swellings 0.5 to 1 cm. in diameter and necrosis of the deep layers of the skin. The swellings persisted for several weeks.

The soluble toxins which Beveridge demonstrated were certainly not very strong. Scrivner and Lee (29), on the other hand, prepared filtrates from pure cultures of Bacterium necrophorum strains of animal origin and found that they did not contain sufficient toxin to affect rabbits injected subcutaneously or intraperitoneally. Furthermore, they failed to find a toxin sufficiently strong to affect calves when the filtrates were injected subcutaneously. They found that immunization with a filtrate was of questionable value in protecting rabbits and calves from artificial infection with the organism.

Filtrates from human strains of Bacterium necrophorum when injected intravenously into rabbits may give rise to some loss in weight, but the toxicity of such filtrates is not very marked. No satisfactory immunity is built up against infections with this organism. Rabbits immunized with human strains develop abscesses when injected with living cultures just as readily as non-immunized animals. With strains of animal origin, vaccination has been unsuccessful (28).

The various strains of Bacterium necrophorum do not form a homogeneous agglutinating group, such as occurs in the case of Eberthella typhosa. Many of the strains have agglutinogens which are unrelated, so that a single agglutination test is insufficient for identification of these organisms (24, 28, 30, 31).

Bacterium necrophorum infections in man. Numerous reports have been made of infections of man with this organism. Schmorl (19) and one of his assistants each developed a small
abscess on one finger while working with their *Streptothrix cuniculi*. Harris (32) described an anaerobic organism, *Bacillus mortiferus*, that he isolated from a liver abscess in man. *B. mortiferus* has many of the features of *Bacterium necrophorum*. Norris (33) found an organism resembling *B. necrophorum* in a liver abscess of a man. This organism was associated with anaerobic cocci, the colon bacillus and *Proteus vulgaris*. No pathologic condition of the intestine was reported in either of the liver abscess cases. This does not exclude the possibility that lesions were present in the colon at the time of the entrance of the emboli into the blood stream.

In 1910 Stemen and Shaw (34) described an acute infection of the skin in a patient who was a government meat inspector. While dissecting an ulceration on the lip of a sheep, the patient had scratched his hand on one of the sheep's teeth and subsequently developed an infection of the hand from which *B. necrophorum* was isolated. Shaw (34) isolated *B. necrophorum* in apparently pure culture in pus from a patient with a lung abscess. Cunningham (35) studied two cases which came to autopsy. In one case, *B. necrophorum* was isolated from abscesses and necrotic tissue of the hip joint, lung infarcts, and blood. There was a 15 cm. bluish hemorrhagic ulceration in the lower part of the ileum which was thought to be the portal of entry of the organism. In the other case, it was found in a retropharyngeal abscess with gangrene and extension to the peritracheal and subcutaneous tissue and mediastinum. There were submucous hemorrhages into the ileum. Harris and Brown (36) isolated an organism which they named *Actinomyces pseudonecrophorus* from the uteri of women with puerperal infection. Their strains did not produce spreading necrosis when injected subcutaneously into rabbits.

In 1934 Shaw and Bigger (37) described a case of necrobacillosis of the lung following an upper respiratory infection, in which the organism was found in a surgical specimen in pure culture. Henthorne, Thompson and Beaver (16) found *B. necrophorum* (*Bacteroides funduliformis*) in pure culture from four liver abscesses, three of them from patients with carcinoma.
of the rectum. They also isolated it in pure culture from a fecal (?) fistula in a patient with carcinoma of the sigmoid flexure and in mixture with other organisms from a patient with a pulmonary abscess.

No attempt has been made to review all of the reported cases, but rather to point out the various types of lesions in which these organisms are found. In France there has been considerable activity among clinicians and bacteriologists in recognizing these infections, as evidenced by the reviews of Teissier (38), Pham Huu Chi (39), and Lemierre (40). In Germany, Brunner (41) has reviewed the literature, and described three cases of his own in which "Bacillus funduliformis" had caused pleural empyemas.

The author has studied strains of Bacterium necrophorum from lesions in many parts of the body, such as in chronic ulcerative colitis and cancer of the rectum, iliopsoas abscess, subacromial abscess, chronic fistula draining from the breast, and osteomyelitis of femur following middle ear infection. It was also isolated from the blood stream of a child who had a severe angina and developed lung abscesses.

The rôle which B. necrophorum plays in chronic ulcerative colitis is not clearly understood. From a group study made over the past eight years the following information has been obtained (26). When the seriously diseased colon is isolated from the fecal stream, as by end ileostomy, aerobic organisms are greatly reduced in number from the colon discharges, the flora becomes almost entirely anaerobic and B. necrophorum predominates (23, 24, 43). Such an isolated colon often remains diseased for years with intermittent periods of quiescence and exacerbation. During periods of quiescence B. necrophorum usually disappears, only to become plentiful again with each new exacerbation. This organism has been found in the great majority of cases of typical ulcerative colitis when appropriate methods for its detection have been used, but it is not found in the normal colon. It is pathogenic for rabbits, producing in them local abscesses and systemic infection, and also for man as indicated by its isolation in pure culture from liver abscesses, from persistent purulent sinuses, from empyema thoracis, and
from a portal thrombus in a patient who died of ulcerative colitis. Recently a strain was isolated in mixed culture with an anaerobic coccus from a lymph node in the mesocolon in a surgical specimen from a patient upon whom a colectomy was performed. Specific antibodies for this organism have been found in the blood in cases of chronic ulcerative colitis and not in the blood of normal individuals, indicating (43, 30) that the organism is implicated in some way in the mechanism of the disease, either as a cause or as a secondary invader.

From this summary of *Bacterium necrophorum* it is evident that the organism is probably a normal inhabitant of the mucous membranes of man and animals. This is further suggested by the fact that necrotic lesions have been experimentally produced in the colon of monkeys, following which the organism has been isolated (21, 23, 24), whereas they were not found in the normal colon. The fact that *B. necrophorum* has not been found in the normal colon does not indicate that it is not present there, but probably that it is present in insufficient numbers to be detected. The strains in general are not highly pathogenic, although once metastatic abscesses or blood stream invasion occurs the mortality is high. It is important that this bacterium be considered when dealing with pus which is foul smelling or when studying cases of a septic nature where aerobes are not found.

More study needs to be given this group of bacteria, since little is known regarding their metabolism (44). It is desirable that technics be worked out and put into general use so that the results of various investigators may be adequately compared.

*Bacteroides furcosus*

Although McCoy and McClung (2) list 15 references for this organism, a review of these references reveals that some authors were merely repeating Veillon and Zuber's (5) description and showing wherein their strains agree or disagree with the original description. According to Veillon and Zuber, this bacillus is rare and is distinguished principally by its morphology. It appears in pus as a very small rod, terminating in two little branches which give it a Y shape. In culture it forms rods, but
many elements are elongated and divide at one extremity into two branches that end in a swelling or knob; others bear branches which in turn subdivide. The bodies of the bacilli and the ramifications are never very long. The round, or more often pear-shaped swellings are numerous. This bacillus is scarcely larger than *Mycobacterium tuberculosis*, is not motile and is gram-negative. Colonies appear only after 3 or 4 days at 37°C., and not at all at room temperature.

On the surface of agar the colonies are fine, form little gray dots hardly raised above the medium, and remain separate and very small. When magnified they appear as little yellowish masses, transparent at the edges, and very finely granular. Within the agar the colonies are so fine and so transparent that one can scarcely see them; under the microscope they are round, yellowish, with thin, regular edges. They never become large, even when they are well isolated.

In broth the culture forms a fine precipitate. This bacillus does not give off enough gas to make any appreciable bubbles, but it yields a sour, slightly fetid odor. Development is slow but the cultures remain alive 15 to 20 days.

Guinea pigs inoculated under the skin develop abscesses from which they generally recover; some die of cachexia after several weeks.

The foregoing description by Veillon and Zuber (5) does not clearly distinguish these organisms from the *Bacterium necrophorum* group. However, the pear-like swellings in cultures are typical of *Bacteroides furcosus*, and the Y-shaped forms in pus are not characteristic of *B. necrophorum*; but in view of its great pleomorphism it would not be unusual to expect to find such forms in pus. Prévot (3) has named this organism *Ristella furcosa*. Cohen (15) claims to have found it in 2 of 16 specimens from lung abscess. He reports that it produced gas and a fetid odor in Smith-Noguchi medium. His statements are somewhat contradictory regarding dextrose fermentation, since he states:—"Gas was not produced in broth or in dextrose broth. Gas was produced in dextrose, maltose, saccharose and mannite." Lactose and inulin were not fermented. Milk was not coagulated
and gelatin was not liquefied. Aside from the appearance of the organisms in pus, the other properties are not sufficient to distinguish this species from *Bacterium necrophorum*.

**Bacillus fusiformis (Fusiformis fusiformis)**

This species is referred to by Prévo1 under the generic name *Fusiformis* given by Topley and Wilson (14). The literature concerning this organism is well reviewed by Weinberg et al. (4).

Isolation of *Bacillus fusiformis* is rather easily accomplished. It is usually found in mixture with many other bacteria, so that it is desirable to streak out adequately on a suitable medium the specimens to be examined. Dilution in fluid media and pour plates involves too much exposure to oxygen for successful isolation. In our experience these organisms may be isolated on 10 per cent sheep-blood agar plates incubated under anaerobic conditions as previously described. Slanetz and Rettger (45) have found that gentian violet at 1:10,000 dilution in 5 per cent blood-agar or 1:20,000 dilution in potato-extract agar permitted good growth of the fusiform bacteria and inhibited heterogeneous types. These workers found that carbon dioxide in an anaerobic environment was satisfactory for good growth of these organisms. This has also been the experience of the author. The colonies are small, and on blood-agar plates a greenish zone of hemolysis may be seen about them.

Morphologically this group of bacteria varies considerably. In lesions, the fusiform bacilli are associated with spirilla. Tunnicliff (46) grew cultures on agar containing ascitic fluid, and in the old cultures found spiral forms. Subsequently (47), she considered the spirilla and fusiform organisms as two phases in the developmental cycle of the same organism. Later (48), she studied the smooth and rough colonies and found the straight forms associated with the smooth colonies, whereas the spiral forms were much more numerous in the rough colonies.

Varney (49) studied 18 cultures from various sources and separated them into four different types based on morphological and serological differences. His types 3 and 4 could be identified
by morphology, whereas types 1 and 2 varied greatly in size and shape and could be differentiated from each other only by serological tests. There seems to be no correlation between Varney's four types and the following.

Slanetz and Rettger (45) have divided these organisms into four groups based on their morphological, cultural and biochemical characteristics. Morphologically they may be distinguished as follows:

"Type I occurs as single cells and in pairs. The ends are definitely pointed. In young cultures they vary in length from 3-6μ, and in width from 0.4-0.6μ. They remain fairly uniform in size, even in old cultures, differing from the other types in this respect. They often contain one or two granules. The cells are shorter than those of any of the other types, and they can often be identified by their morphological appearance."

"Type II is long and slender, often growing in long filaments. The ends are definitely pointed, as a rule. The shorter forms vary in length from 6 to 20μ and in width from 0.3 to 0.6μ. Numerous granules are present in old cultures."

"Types III is thicker and often longer than type II. Chains can frequently be observed. They measure from 6-25μ in length and from 0.6 to 0.8μ in width. The ends are only slightly pointed. In old cultures the cell outline fades away and granules develop."

"Type IV cells are usually larger than those of the other three types. They occur in characteristic chain formation, and it is often difficult to distinguish between individual cells and a chain of cells. They vary from 8 to 25μ in length, and from 0.7 to 1.0μ in thickness. Granules appear in old cultures. On agar medium containing 25 per cent carrot extract the cells increase in size, and numerous granules develop after 48 hours incubation. They present an entirely different morphological appearance on this medium than when growing on potato extract agar."

These authors found that types I and IV could usually be identified by morphology, whereas II and III were difficult to separate in this way but could be separated by other tests. Spiral forms were not found in types I, II and III, but a few were formed in type IV which appeared to develop from filaments or from deep staining bodies within the cells, as described by Tun-
Nicliff. Colony types could not be distinguished on blood-agar but could on potato-extract agar. They detected no difference in the types from their growth in broth media.

Gelatin is not liquefied by strains of *B. fusiformis*. Slanetz and Rettger (45) studied the fermentation of glucose, sucrose, lactose and mannitol. Their type I and II strains ferment glucose only, type III glucose and sucrose and type IV glucose, sucrose, and lactose; but none ferment mannitol. None produce gas, which is in accord with the results of most investigators, although Prévot (3) reports that very little gas is produced in glucose-agar with serum. The latter also states that mannitol is fermented by most strains. Milk is coagulated but not digested.

Although Rosenow's brain medium is used for the cultivation of many of the non-sporulating anaerobes, in our experience the fusiform bacilli fail to grow in this medium without the addition of blood or serum. A fetid odor is produced when growth occurs.

Although Varney (49) has been successful in separating members of this group by their agglutination reactions, most other workers have failed. In this connection, Slanetz and Rettger (45) make the following statement: "It was frequently difficult to distinguish between specific and spontaneous agglutination. Furthermore, no definite correlation between the agglutination reaction and the type of organism could be established. Most of the strains were either agglutinated by all of the antisera, or did not react definitely with any of them."

**Pathogenicity for man.** Weinberg et al. (4) credit Miller (50), rather than Plaut (51) or Vincent (52), as the first to observe fusiform bacilli in ulcerative stomatitis. Since then (1890), many investigators have found this organism in a variety of ulcerative processes. The name of Vincent is frequently applied to one type of infection, namely Vincent's angina. Vincent (52) published a number of papers on this subject. In the first, which appeared in 1896 and was concerned with hospital gangrene, he described *B. fusiformis* as rectilinear or incurving, frequently filamentous, with the extremities pointed and gram-negative. He noticed the formation of granules or vacuoles, the frequency
of involution forms, immotility and absence of spores. In 40 of 47 cases spirilla were associated with the bacilli.

The fusiform bacilli have been found in normal throats and in ulcerative processes involving the mucous membranes of the throat, colon and vagina, also in noma and lung abscesses. In our laboratory we have frequently encountered them in the ulcerated colon.

The interesting question arises as to why stomatitis (trench mouth) and Vincent's angina are not more prevalent. The factors which contribute to natural resistance against these organisms are not understood. Wallace, Wallace and Robertson (53) have shown that daily intravenous injection of 0.25 lethal dose of scillaren B, a squill glucoside, induces a typical clinical picture of Plaut-Vincent's angina in the dog. Typical fusiform bacilli and spirilla were found in smears from the lesions. When the injections were discontinued, some of the dogs recovered.

Lichtenberg, Werner and Lueck (54) found fusospirochetal organisms in about 45 per cent of tonsils removed from 108 children. They observed these organisms in 91 per cent of the membranes that formed over the tonsillar beds after tonsillectomy, and usually in greater numbers than in the tonsils themselves. Sixteen consecutive cases of severe ulcerative stomatitis in children healed in some 4 to 7 days without treatment, which they found to compare favorably with the reports of cases treated with various drugs and other forms of therapy. Recently (55) nicotinic acid has been reported to be a specific therapeutic agent in stomatitis, which would suggest a metabolic disturbance in the host as responsible for infection with these organisms. King (55) inoculated his own mouth with infected material from a severe case of Vincent's disease and failed to induce inflammation or ulceration. The organisms grew in abundance for a short time but disappeared three days after inoculation.

**Bacterium melaninogenicum**

This organism is particularly interesting due to the coal-black appearance of the colonies which develop on anaerobic blood-
agar plates. Prévot (3) has placed this organism in the genus Ristella. Bacterium melaninogenicum, Bacteroides melaninogenicus and Ristella melaninogenica are synonyms.

Oliver and Wherry (56) described and named this organism. They cultured it from the mouth, tonsils, infected abdominal wounds, focal infection of the kidneys, the feces, and from the stools of patients with chronic amebic dysentery. Considerable study has been given this organism by Burdon (57), who describes it as a very small, non-spore-bearing, gram-negative anaerobic diplococcobacillus. Its growth in pure culture is feeble, but it grows readily in mixture with other bacteria with which it is found. Mixed anaerobic blood-agar cultures are characterized by a very extensive destruction of hemoglobin, the formation of large amounts of a brownish-black melanin-like pigment, and the production of a foul odor. The pigment develops slowly so that the characteristic black colonies on blood-agar may not be obvious until after 4 or 5 days. The pigment is similar to but not identical with melanin. In culture the organism has marked proteolytic powers, causing rapid digestion of coagulated serum and other native proteins. Burdon (57) questions the purity of the cultures of Oliver and Wherry (56), since the reactions reported by them were similar to those which he obtained in mixed cultures. B. melaninogenicum grows in the same colonies with other organisms and is difficult to isolate and maintain in pure culture. Oliver and Wherry (56) did not use plating methods for the isolation of their cultures.

Burdon (57) examined the anaerobic blood-agar slant cultures from 5 cases of uterine infection studied by Schwarz and Dieckmann (9, 10) and identified B. melaninogenicum as the pigment-producing organism in them. Schwarz and Dieckmann (9) had found that puerperal fever, (of the type doubtless due to auto-infection), frequently involving the pigment-producing organism, is extremely rare in patients observing good personal hygiene and occurs most commonly in the less cleanly colored ward patients. Whether Bacterium melaninogenicum is a true pathogen or a secondary invader is still unknown.
Bacillus nebulosus

This organism was described by Hallé (20). It is briefly discussed by Weinberg et al. (4) in a chapter entitled "Insufficiently described gram-negative bacilli." It is a small bacillus resembling the bacillus of mouse septicemia (32). Usually straight, it sometimes is curved, appearing as a rod swollen at the center and tapering at the extremities. It is gram-negative, non-sporulating and shows no involution forms. Growth at 37°C. is slow, and no growth is obtained at room temperature. No gas is formed in sugar media. It is inconstant in its pathogenic properties and occasionally produces abscesses in rabbits and guinea pigs.

Bacterium pneumosintes

This bacterium has been placed in the Family Ristellaceae by Prévot (3) and in a genus Dialister, which he defines as including very small, non-motile, gram-negative, non-sporeforming organisms which pass through Berkefeld V and Chamberland L2 filters. Olitsky and Gates (58 to 62) first isolated these organisms from the nasopharyngeal washings of patients in the early stage of influenza. The cultural characteristics are well summarized by Topley and Wilson. This species may be cultured in Smith-Noguchi medium (human ascitic fluid containing a piece of sterile rabbit kidney and covered with a vaseline seal). After 3 to 4 subcultures in this medium, it will grow anaerobically on blood-agar, chocolate agar and Bordet's medium. Morphologically the organisms are minute bodies, arranged singly, in pairs, or short chains; the length varies from 0.15–0.3 μ and the breadth from one-half to one-third of the length. The center stains more deeply than the ends.

On horse blood agar, the colonies after 7 days' incubation at 37°C. are round, convex, milky-white, opaque, and measure about 0.5 mm. in diameter. They are amorphous with a smooth glistening surface and an entire edge; there is no hemolysis. Following incubation at 37°C. for 5 to 7 days in Smith-Noguchi medium, they remain viable at room temperature for two and a half years. The organisms withstand freezing and drying in
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vacuo, and remain viable for a long time when dried. Organisms in infected rabbit lungs kept in 50 per cent glycerol at 4°C. survive for 9 months, during which time the virulence is maintained. At 56°C. for 30 minutes the organisms in the moist state are destroyed. They pass through Berkefeld N and V filters.

Acid is produced from dextrose; indole, nitrite and catalase are not produced, and methylene blue is not reduced. Ag-glutinins are formed following injection of cultures into rabbits. Injection of mass cultures intratracheally into rabbits produces a rise of temperature in 24 hours and sometimes a conjunctivitis and a mononuclear leucopenia. Recovery occurs in 2 to 3 days. If the rabbit is killed during the acute stage of the illness, edema and emphysema are found in the lungs. Numerous hemorrhages, discrete or diffuse, are seen on the surface of the lungs; but the pleura is not involved. On section of the lung a frothy blood-stained fluid escapes and hemorrhages are found scattered through the parenchyma. A muco-purulent exudate is present in the trachea and bronchi. These organisms are non-pathogenic to monkeys when injected intratracheally.

Mills, Shibley and Dochez (63) found these gram-negative filter-passing anaerobes in individuals throughout the year, and for that reason consider that no causative rôle can be assigned to them in the etiology of influenza or the common cold.

Bacillus ramosus

This organism is known under several other names, such as Bacteroides ramosus, Fusiformis ramosus and Ramibacterium ramosum. Prévot (3) has placed it in the Family BACTERIACEAE and has given the generic name Ramibacterium to rods straight or curved, non-sporulating, non-motile, not ciliated, not encapsulated, gram-positive and presenting pseudo-branching. B. ramosus was first described by Veillon and Zuber. A translation of their description follows.

This bacillus is as constant as B. fragilis in pus of appendicitis, but it seems less abundant. It appears identical with B. ramosus encountered in pulmonary gangrene. In pus it occurs as a small fine rod, not as long as the tubercle bacillus, and is either isolated or grouped in
clusters. In culture, the rods have the same form as in pus, but a large number of them are somewhat larger and especially longer; some assume irregular forms and are straight or concave; the ends are sharp and the thickness is variable; some contain swellings. Some cells are united two by two in a V shape; others are isolated. Certain rods are branched, and a quite long bacillary form may be seen which is divided at one end into two little branches like a V; others bear several of these little branches throughout their length. In some several branches seem to shoot off from one swelling. The bacillus is non-motile, gram-positive, grows only at 37°C., and requires 3 to 4 days' incubation for good growth. On gelatin no growth is observed. In deep agar the colonies are round or oval, granular, brownish-yellow, at first with smooth edges which later appear bristling with very fine short filaments. On the surface of agar the colonies are very small, gray-white and transparent. Broth is uniformly clouded in 3 or 4 days and forms a muddy, grayish mass. A little gas and a sour fetid odor are given off. Cultures remain viable for about a month; these organisms are non-sporeforming.

When \textit{B. ramosus} is injected into guinea pigs subcutaneous abscesses are formed; in rabbits abscesses are formed and the animals die in 8 to 10 days. Intravenous injection into rabbits causes the death of the animals in several days and gives rise to intoxication and cachexia.

This description by Veillon and Zuber, although incomplete, is very useful in the isolation and identification of \textit{B. ramosus}. Other characteristics of this species are added in the review of the literature by Weinberg et al. (4). In peptone-water growth is meagre and indole is not produced, gelatin is not liquefied, and milk is coagulated. Acid is formed in glucose, maltose, galactose, sucrose, mannitol and lactose. No hemolysis occurs on blood-agar.

Weinberg and Prévot grew \textit{B. ramosus} in glucose broth culture for 24 to 48 hours, and then centrifuged the culture. When 3 ml. of the supernatant fluid was injected intramuscularly into guinea pigs, pain was produced in the part injected; dyspnea and respiratory paralysis followed, after which the heart continued to beat for a short time. Upon injection of a sub-lethal dose (1 to 2 ml.) the muscles went into spasm at the site of injection, dyspnea developed and then gradual recovery followed. The toxin is not hemolytic either \textit{in vitro} or \textit{in vivo} and is not precipitated.
with ammonium sulfate. Antigenicity was not determined because of the transient nature of the toxin which, together with virulence, was lost after 6 weeks to 2 months of cultivation. Agglutinins were obtained for homologous strains.

Pathogenicity for man. *B. ramosus* has been found in numerous infections in man, such as mastoiditis, chronic otitis, pulmonary gangrene, putrid pleurisy, cavernous tuberculosis and gangrenous appendicitis. It is sometimes found in infections of the urinary tract, intestinal ulceration, liver abscess and osteomyelitis.

Lemierre, Reilly and Bloch-Michel (64) have reported five cases of *B. ramosus* infection observed at the Claude-Bernard Hospital, Paris. The first was one of gas gangrene due to *B. ramosus* and aerobic hemolytic streptococci following a hypodermic injection. The patient recovered after incision and drainage of the wound. In the four other patients *B. ramosus* was isolated from the blood. In one of the latter patients the fact that the blood-culture was positive only once out of five times raises the question as to whether the bacillus might have been only a transitory invader. In the three others, whose blood-cultures were positive, the authors were inclined to believe that the organism was a secondary invader. Compared to the gravity of infections due to *Bacterium necrophorum*, those due to *B. ramosus* are relatively benign.

*Bacteroides serpens*

This organism has been classed in the Family ristellaceae and in a genus *Zuberella* by Prévot (3), who defines the genus as containing: Straight rods, non-sporulating, gram-negative, motile, ciliated and not encapsulated.

This organism is known under the names *Bacillus serpens*, *Bacillus radiiformis* and *Zuberella serpens*. The species was first described by Veillon and Zuber (5) as a small rod, quite bulky, regular, with rounded ends. In cultures, the cells are often united two by two or form pseudo-filaments. It is slightly motile and progresses especially by undulation. It develops between 20° and 37°C. Gelatin is liquefied. In agar at 37°C. at the end of 24 hours little colonies appear. When magnified,
they look like little round masses, clear, grayish, granular and shaded, and sometimes a bunch of threads appears at one of the poles. Later the colony, growing larger, becomes more opaque and the edges more clearly defined.

On the surface of anaerobic plates little dots appear which are scarcely visible at the end of 48 hours; later the colonies form little cloudy masses which are transparent. Broth becomes very turbid during growth and then clears, leaving a white sediment in the bottom of the tube. The cultures give off a fetid odor but deep agar is not broken. The cultures remain viable for 20 to 25 days. Bacteroides serpens is gram-negative and strictly anaerobic.

The cultures are pathogenic to the mouse, guinea pig, and especially the rabbit. Inoculated under the skin, they produce abscesses, and the animals die of cachexia at the end of 7 to 8 days. Pus containing this organism in mixture with others is more virulent than the pure cultures alone. Veillon and Zuber (5) obtained their culture of Bacteroides serpens in mixture with B. ramosus from a child with a mastoiditis, who was operated upon and died 24 hours later. At autopsy an otitis media, gangrenous abscess in the sphenoidal lobe and gangrenous foci in two lobes of the lungs were found. The abscesses in the lungs and the one in the brain contained foul-smelling pus.

Prévot (3) lists the following additional characteristics of Bacteroides serpens. Clouding occurs in peptone-water. No indole is produced. Milk is acidified, then coagulated and gas is given off. Brain medium is blackened. Acid and gas are produced in glucose, levulose, maltose, galactose and lactose broths. No toxin or hemolysin has been demonstrated.

DISCUSSION

In this review only the non-sporulating anaerobic bacteria of medical importance have been considered. Those of non-medical importance are little known, even though in the intestinal tract they may outnumber Escherichia coli (14). There are many reasons why our knowledge of this group of anaerobes is so meager, although the organisms have been encountered in a wide
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variety of lesions. In the first place, they have been assigned names and characteristics on the basis of inadequate study. Furthermore, medical bacteriologists know practically nothing about the group, and their attempts to inform themselves have led more to confusion than to systematic knowledge. In this review only those organisms which have been most studied are considered, and of 266 species only 4 members of the coccus group (Streptococcus anaerobius, S. foetidus, S. putridus and Staphylococcus parvulus) and ten of the rod-shaped organisms were selected from the bibliography of McCoy and McClung (2). Of the 10 members of the bacterium group two may be the same species (Bacterium necrophorum and Bacillus funduliformis) and of the remaining eight only four have been studied to any extent (Bacteroides fragilis, B. fusiformis, Bacterium pneumosintes and Bacillus ramosus). Not more than 18 references have been listed (2) for any of the remainder (Bacteroides furcosus, Bacterium melaninogenicum, Bacillus nebulosus and Bacteroides serpens).

One characteristic of all of these organisms is that they are associated with ulcerative processes involving the mucous membranes and that under certain circumstances they may invade the tissues and produce abscesses from which foul-smelling pus is obtained. They are frequently found in the blood stream in septicemias. An effort should be made on the part of teachers in medical schools to inform students of this group of organisms and to teach those in laboratories of medical bacteriology to be aware of and to recognize them. The author apologizes for using the assortment of generic names commonly applied to these bacteria and listed in the subject bibliography of McCoy and McClung. It would seem better to use the generic name Bacterium for the non-sporoforming rod-shaped species until such a time as a suitable classification may be given. Prévot is to be complimented on his attempt to classify them. The difficulty with his classification is that he has accepted inadequate descriptions of organisms and thus increased the number of species. A careful study of this group of bacteria, using uniform methods, would eliminate the species which were created on the basis of inadequate study.
REFERENCES

(6) König and Menge 1897 Bakteriologie des weiblichen Genital-Kanals Leipzig.
(13) Personal communication with Dr. Robert Breed.
NON-SPOREFORMING ANAEROBIC BACTERIA


(27) McCullough, N. B. 1938 Vitamin C and resistance of the guinea pig to infection with Bacterium necrophorum. J. Infectious Diseases, 63, 34–53.


(40) LEMBERT, A. 1936 On certain septicæmias due to anaerobic organisms. Lancet, 1, 701-703.
(48) TUNNICLIFF, R. 1933 Relation of spiral organisms to the rough colony of Bacillus fusiformis. J. Infectious Diseases, 53, 280-286.
(49) VARNEDO, P. L. 1927 The serological classification of fusiform bacilli. J. Bact., 13, 275-314.
(50) MILLER. 1890 Die Bedeutung der Mikroorganismen der Mundhöhle für den menschlichen Organismus. Prager med. Wochschr., 34, 475.
NON-SPOREFORMING ANAEROBIC BACTERIA