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# The Genus *Alcaligenes* and Related Organisms

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THE GENUS *ALCALIGENES* AND  
RELATED ORGANISMS

by

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A Dissertation Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of  
the Requirements for the Degree of  
Doctor of Philosophy

1953

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## CHAPTER I

### INTRODUCTION AND STATEMENT OF PROBLEM

Petrushky, a German bacteriologist, in 1889 published a report concerning an organism isolated from spoiled beer which he believed to be a hitherto unknown type. In 1896 he published a more extensive report on what he stated to be the same organism and described it in more detail. He named the organism Bacillus faecalis alcaligenes, and stated its identifying characteristics. Unfortunately some of Petrushky's cultures were later found to be impure and much controversy arose over the morphology and to some extent the physiology of the organism originally isolated. In the course of time it became increasingly clear that there existed a number of morphological types of organisms having physiological characteristics similar to the organism described by Petrushky.

This investigation was undertaken to determine the flagellar morphology and the biological characteristics of organisms placed in the genus Alcaligenes by contemporary bacteriologists. Organisms related to Alcaligenes were also studied.

## CHAPTER II

### HISTORY

#### Alcaligenes faecalis

Petruschky (1889) investigated some spoiled beer and isolated an organism from an agar plate streaked with the beer. The organism was described as a short gram negative rod with lively motility. Potato growth was yellowish in 48 hours and later the potato became brownish. He could differentiate the beer bacillus from the typhoid bacillus by his litmus whey medium which the typhoid bacillus turned acid while the beer bacillus turned it alkaline. The colonies on agar, of the beer bacillus and the typhoid bacillus, were quite similar, as was also their action in gelatin which was not liquefied.

Gunther (1894) isolated a comma shaped bacillus from soil which he named Vibrio terrigenus. It was identical in size and shape with the cholera vibrio. The organism was described as being motile with a tuft of polar flagella on both ends. There was no liquefaction of gelatin. This strict aerobe did not ferment sugars and gave a yellowish brown growth on potato. Milk was not coagulated and the nitroso-indol reaction was negative.

Petruschky (1896) described an organism which, according to him, was the same as that isolated by him from spoiled beer (1889) and described it as a typhoid-like alkali producer. This organism was found in stool specimens. It was described as being gram negative, actively motile with

peritrichous flagella. (Photomicrographs did not accompany this report.) Colonies on gelatin were similar to those of the typhoid organism. Milk cultures became alkaline without coagulation. No gas was produced in sugar media (the nature of the sugar was not stated nor was anything said concerning acidity). He could differentiate the Bacillus faecalis alcaligenes (n. sp.) from the typhoid bacillus by the following reactions: a) alkalinity in 48 hours in litmus whey, b) no agglutination with typhoid serum, c) thick layer of growth on potato, with subsequent browning of potato. Milk was also found to become alkaline in 48 hours. Intraperitoneal injection of animals caused infection while subcutaneous injection did not.

Flügge's (1896) taxonomy listed the organism as Bacillus faecalis alcaligenes (Petruschky), and Chester (1897) in his classification of Schizomycetes also retained the trinomial but changed the genus and altered the spelling to Bacterium faecalis alcaligenes (Petruschky).

Fischer (1899) reported the isolation at autopsy of Bacillus faecalis alcaligenes from various organs of a child with measles and tuberculosis. He also found the organism in sewage disposed in canal water.

Lehmann and Neumann (1899) renamed the organism Bacterium alcaligenes (Petruschky). Migula (1900) named it Bacillus alcaligenes (Petruschky) Mig.

Ford (1901), in attempting the classification of intestinal bacteria, recognized a group of alkali producing bacteria which at this time he separated into Petruschky groups 1, 2, and 3. They were collectively described as asporogenous rods producing an alkaline reaction in milk without a preliminary acidity and no coagulation. They failed to liquefy gelatin, casein or coagulated blood serum. They produced no gas from dextrose, lactose

or sucrose and indol was not produced. (There is no mention of gram reaction, flagellar morphology or acid production from carbohydrates.) Ford was not certain if group 1 or group 2 was identical with Petruschky's original bacillus or whether both were varieties of this bacillus. The groups are differentiated in the table below.

alkali producers		motile	grows with scum on broth	potato growth luxuriant and visible	growth in closed arm of Smith tube	nitrate reduced to nitrite
Petruschky groups	1	+	+	-	-	-
	2	+	-	+	+	+
	3	-	-	-	+	+

Petruschky (1902) found Bacillus faecalis alcaligenes in pure culture in 6 rose spots on a patient with clinical typhoid fever. He was of the opinion that the organisms isolated earlier from stools and those isolated from rose spots were identical. He also stated there was a natural typhoid-like disease not caused by the typhoid bacillus but apparently by Bacillus faecalis alcaligenes.

Ford (1903) in his detailed study of the classification of the intestinal bacteria in man followed Migula's (1900) taxonomy except for manifest errors. He considered the correct name to be Bacillus alcaligenes, Migula (1900) and not Bacillus alcaligenes (Petruschky) Migula. He discusses the validity of reactions by which organisms are recognized and stated that he found growth on potato unreliable since potatoes were not of constant composition. He pointed out that the same organism inoculated on two different potatoes may not show the same characteristic growth and that



appearance on potato is not of taxonomic importance. Using the Smith fermentation tube with dextrose broth, Ford distinguished three groups of bacteria. 1) organisms which have no apparent effect on carbohydrates, their growth being limited to the bulb of the fermentation tube where it extended as far as the neck only, the broth in the closed arm remaining clear. The reaction in the bulb is usually alkaline. 2) bacteria which ferment the carbohydrates with the production of acid but no gas, the growth extending throughout the tube. The reaction in the arm is always acid, and in the bulb either acid or alkaline. 3) organisms which show turbidity and gaseous fermentation in the closed arm. Bacillus alcaligenes was recognized as belonging to group one. This group was further subdivided as shown in the table below. There is some discrepancy between Ford's table and his detailed

	motility	scum on broth	potato growth luxuriant and visible	growth in closed arm	NO <sub>3</sub> to NO <sub>2</sub>	gelatin liquefied	casein digested	coagulated blood serum liquefied	milk		
									acid	coagulated	alkaline
<u>Bacillus alcaligenes</u>	+	+	+	-	-	-	-	-	-	-	+
<u>Bacillus recti</u>	+	-	+	-	+	+	-	-	+	-	+
<u>Bacillus pylori</u>	+	-	+	-	+	+	+	-	+	-	+
<u>Bacillus casei</u>	+	+	+	-	+	+	+	+	+	-	+
<u>Bacillus Bookeri</u>	+	-	+	-	-	+	+	+	+	-	+

description of the individual organisms. In the table above the milk reactions

for recti, caeci and bookeri are listed as producing an acidity and alkalinity, however in the detailed description of the individual organisms there is no mention of preliminary acidity. (This production of an initial acidity, probably from dextrose in the milk, and subsequent alkaline reaction is perhaps analogous to the similar reaction occurring with the Shiga bacillus and Proteus vulgaris. None of the organisms in the above table grow in the closed arm of the Smith fermentation tube yet Ford described them as facultative anaerobes.)

Altschuler (1904) was of the opinion that the typhoid bacillus could change to Bacillus faecalis alcaligenes and vice versa. Doebert (1905) using 3 strains of Bacillus faecalis alcaligenes, two of which were from Petruschky, came to the same conclusion. Doebert found a serological cross reaction between his three strains and the typhoid bacillus.

Berghaus (1905) used Doebert's Bacillus faecalis alcaligenes I (Strain II of Petruschky) and found it to be a mixture of: 1) plump rods with peritrichous flagella which "moved like ants" in a moist preparation. 2) slender rods with a polar flagellum which moved in a straight line. (Photomicrographs did not accompany the report.) The two organisms were separated and identified serologically and by litmus milk as the typhoid bacillus and Bacillus faecalis alcaligenes respectively. Using the pure cultures Berghaus could not transform one into the other, nor could he demonstrate any cross agglutination. Berghaus found Bacillus faecalis alcaligenes to be a strict aerobe, growing only in the open part of the Smith fermentation tube.

Trommsdorff (1905) recognized that the Bacillus faecalis alcaligenes

used by Altschuler and Doebert, which they claimed were transformable into the typhoid bacillus, originated from Petruschky. He obtained from Ficher in Berlin transplants of Petruschky's strain AI and Doebert's strain AII. Their cultures were plated and colonies were fished. Subsequent cultures were typical for Bacillus faecalis alcaligenes and gave no agglutination with typhoid serum. Doebert's transformation experiments were repeated with negative results. The author concluded that Bacillus faecalis alcaligenes is a group of organisms rather than a single species, and that the organisms cannot be transformed into the typhoid bacillus.

Conradi (1905) agreed with Trommsdorf for he also found Altschuler's cultures were contaminated.

Berghaus (1905) after extensive study of seven strains of Bacillus faecalis alcaligenes came to regard this organism as a nonpigment producing strain of Bacillus fluorescens non-liquefaciens. He observed the motility in his collection to be a linear motion, unlike the wiggling motion of typhoid bacilli. Berghaus could not confirm Petruschky's statement that Bacillus faecalis alcaligenes has peritrichous flagella for even Petruschky's strain II had 1 to 2 polar flagella. One of the several strains studied had lophotrichous or polar multitrichous flagella. The Zettnow method was used and was found to be superior to the Loeffler technique for staining flagella. Growth on potato was brown, and all were strict aerobes. Pellicle formed on broth and there was no effect on dextrose and lactose. Indol test was negative. Nitrates were reduced to nitrites.

Krencker (1905) adds nothing to the already existing description of the organism.

Terburgh (1906) isolated 14 blue colonies from Drigalski-Conradi agar inoculated with canal water. They were identified by Petruschky's litmus whey medium as Bacillus faecalis alcaligenes.

Piorkowski (1906) concluded that the typhoid bacillus and Bacillus faecalis alcaligenes are different on the basis of growth on gelatin. He described colonies of both organisms.

Trincas and Olla (1907) described cases of alimentary intoxication presumably from cheese from which was isolated Bacillus faecalis alcaligenes. The organism was gram positive and produced green pigment.

Gaehtgens (1907) using a freshly isolated typhoid bacillus from the blood of a patient and 3 strains of Bacillus faecalis alcaligenes was unable to transform the one organism into the other by in vitro methods. Gaehtgens noticed differences in growth of the organisms on several media. He also observed that antiserum prepared for one strain did not agglutinate all other strains of Bacillus faecalis alcaligenes.

Klimenko (1907) collected 22 strains of Bacillus faecalis alcaligenes from various sources. Fifteen of these were obtained from laboratories in Russia and Germany, including 3 strains directly from Petruschky. One of Petruschky's strains produced yellow pigment on an agar slant. These 15 strains were carefully compared with 7 of his own strains isolated from feces, urine, and canal water of Petrograd. Klimenko also studied Bacillus fluorescens non-liquefaciens and Bacillus fluorescens putidus Flugge. It appears obvious from the data presented that the organisms studied by Klimenko as Bacillus faecalis alcaligenes were of many types. He suggested that the

organisms be differentiated on the basis of pigment and acid production from carbohydrates. He objected to the suggestion of Berghaus (1905) that

Bacillus faecalis alcaligenes be considered as a nonpigmented Pseudomonas.

Klimenko concluded that Bacillus faecalis alcaligenes was polar flagellated.

Klimenko (1908) described Bacterium mariense (nov. spec.) as a new type of alkali producer. He excluded this organism from the Bacillus faecalis alcaligenes group. The organism was isolated from the spleen and blood of a guinea pig and was motile with peritrichous flagella (photomicrographs were not published), asporogenous and a gram negative rod. It produced no pigment, did not liquefy gelatin and produced an alkaline reaction in milk. Nitrates were not reduced to nitrite. Alkali was formed in 2% carbohydrate broth with litmus indicator. Hydrogen sulfide was produced.

Lafforgue (1908) reported the isolation of Bacillus alcaligenes from the blood of a case evolving like benign typhoid fever. Petruschky's litmus whey became intensely blue. (Flagellation was not reported.) There was fermentation of several carbohydrates with the exception of lactose. The indol test was negative.

Jorns (1908) did not describe the organism but found no catalase in Bacterium alkaligenes. Catalase was present in Pseudomonas pyocyanea.

Symmers et al. (1908) isolated a bacillus from Belfast tap water which resembled Bacillus faecalis alcaligenes and in this paper spoke of it as B. Grosvenor. Wilson (1909) relabeled the organism B. aquatilis alcaligenes. This bacillus came to their attention for it was agglutinated by the serum from practically every case of epidemic cerebro-spinal meningitis in Belfast. Ninety percent of 184 different sera agglutinated the organism in a dilution

of 1:50. Bacillus faecalis alcaligenes (Kral) did not absorb out these agglutinins. B. Grosvenor was described as gram negative and motile. There was no liquefaction of gelatin, no gas (no mention of acidity) in glucose broth or any other sugar or alcohol tested, milk was not coagulated, a faint trace of indol was produced, Petruschky's litmus whey became faintly alkaline and potato growth was brown.

Ridder (1909) isolated Bacillus faecalis alcaligenes from the blood of a patient with food poisoning after having eaten a "pork dish". The organism was described as being motile, no effect on milk, no fluorescens and litmus whey became strongly alkaline. The author was inclined to consider the organism responsible for the clinically typical case of food poisoning, for it was agglutinated by the patients serum.

Hama (1910) isolated Bacillus faecalis alcaligenes from the urine and peritoneal fluid of a patient. The organism was described as producing alkalinity in litmus whey but no coagulation of milk. There was no gas in fermentation tubes containing dextrose, lactose or sucrose. (There is no definite statement concerning acidity in dextrose or flagellar morphology.)

Hillenberg and Bierotte (1910) reported an epidemic of gastro-enteritis involving 33 people in which Bacillus faecalis alcaligenes was isolated from suspected meat. The authors did not consider the organism responsible for the meat poisoning outbreak. It was described as being motile and having the physiological reactions of Bacillus faecalis alcaligenes.

Castellani (1910) reported the isolation on bile salt agar of a gram negative spirillum from stools of fatal human cases of enterocolitis. The name Spirillum seylanicum was proposed. The organism varied greatly in

length and shape. Some individuals were 20 to 40 microns in length with 2 to 4 coils while others were rods or comma shaped. Litmus milk showed no acidity but after 8 weeks the milk was alkaline and peptonised. Broth growth showed a general turbidity with pellicle while growth on agar was whitish like that of colon bacilli. There was no liquefaction of gelatin or coagulated serum. The indol test was negative. There was no change in dextrose, lactose and sucrose litmus broth.

Ferry (1910) isolated an organism from the respiratory tract of dogs. The organism was described as a short narrow motile bacillus. (No name, gram reaction or flagellar morphology was given.) A plain agar stroke in 24 hours showed moderate growth which was moist, glistening, smooth and of a sticky consistency. Plain agar stab growth was best near the surface. Potato growth was a light tan color, abundant, raised, moist, glistening and sticky with a decided odor of stale bread. Potato became slightly darkened. Loeffler's blood serum was not colored or liquefied. Gelatin stab showed no liquefaction. No surface growth in broth, but the medium was moderately cloudy. Litmus milk in 72 hours begins to turn blue at the surface, at five days the whole tube was blue, at fourteen days the color disappeared from the bottom of the tube, but no coagulation occurred. Smith fermentation tubes with dextrose or other carbohydrates showed clouding in the open arm with no visible growth in the closed arm. Neither acid nor gas was produced and the medium became alkaline. Indol was not produced. Serum from dogs suffering from distemper always agglutinated this organism.

McGowan (1911) isolated and described an organism which he found many times in animals with distemper. His description corresponded to that of

Ferry (1910) and added the organisms were gram negative without spores. Motility was never very marked, but flagella were present. (Very poor photomicrographs show flagella very faintly on organisms clumped together.) The author could form no idea of the number or arrangement of the flagella. A pellicle was not formed in fluid media. Litmus milk was alkaline after 24 hours, no coagulation was observed. The organism did not produce acid or gas in peptone salt medium containing glucose, lactose, sucrose or other listed carbohydrates. However, the medium became markedly alkaline. The organism grew readily anaerobically in the depth of stab cultures in glucose agar, and in Buchner's tubes with pyrogallate of soda.

Ferry (1911) reported it was possible to isolate the organism he described (1910) from both the respiratory tract and blood of dogs. He named the organism B. bronchicanis and stated it was gram negative. In 1912 he realized this was not an appropriate name, for the organism could be isolated from other animals, hence he proposed the name Bacillus bronchisepticus.

Kuhnemann (1911) examined numerous strains labeled Bacillus faecalis alcaligenes and found them always to be polar, monotrichous or bipolar, but never with peritrichous flagellation as Petruschky had reported. The author used flagella staining, a modification of Loeffler's technique, routinely to identify organisms. Beautiful photomicrographs show the lophotrichous flagellation of Bacillus faecalis alcaligenes isolated from feces.

Glaser and Hachla (1911) found that the morphology of Bacillus faecalis alcaligenes resembled cholera vibrio. The Kral strain of this organism was found to have fluorescent pigment and was therefore not typical but rather a Pseudomonas. The author found Bacillus faecalis alcaligenes



almost in pure culture in diarrheal conditions in cholera patients. They agree with Klimenko and not with Berghaus regarding the relationship of Pseudomonas and Bacillus faecalis alcaligenes.

Horn and Huber (1911) occasionally found Bacillus faecalis alcaligenes in the feces of healthy cows. The three strains isolated had lively motility and polar flagella, usually unipolar. Moderate growth appeared on agar slants, gelatin was not liquefied, milk turned yellow but without coagulation. Litmus whey became blue in 2 to 4 days. No gas from dextrose and lactose. Indol was negative. No acid or gas from glycerol. Hydrogen sulfide formation was moderate.

Poppe (1911) found, among many other organisms, Bacillus faecalis alcaligenes in the yolk and white of normal hen's eggs. He observed that only motile bacteria go through the shell of the egg. Infection of the egg occurs during its formation in the hen. Fertilised eggs were most often contaminated.

Schottmuller (1911) listed two cases of clinical typhoid fever from which Bacillus faecalis alcaligenes was isolated from the blood. The patient's serum agglutinated the organism isolated. The organisms were described as being gram negative, motile rods, producing red colonies on Endo's medium. (Although this organism did not produce gas from dextrose there is no record of an acid or alkaline reaction in dextrose medium.)

Hanser and Springer (1912) isolated Bacillus faecalis alcaligenes from various organs of a patient with atypical symptoms of typhoid fever. Typhoid bacilli were not isolated from the patient but the serum agglutinated typhoid bacilli to a dilution of 1:200. The isolated Bacillus faecalis

alcaligenes agglutinated in the patient's serum to a titer of 1:200. (The organism was not described.)

Baerthlein (1912), in a study of differential media for cholera vibrio, isolated numerous organisms which morphologically were like cholera vibrio. These types were almost exclusively found in patients with intestinal disease, and one gets an impression of an etiologic relationship of the organism to the disease. In three cases the organisms were found in the blood of the patients. The organisms were curved on Dieudonne's medium but rapidly became quite straight on cultivation on other media and experiments with media failed to change them back to the curved type. The organisms were found to be nonhemolytic. Many serological types were found. The strains produced a raspberry or mulberry like colony on gelatin. Gelatin was not liquefied in 14 days by these organisms. The various strains showed either bipolar or unipolar monotrichous flagellation. Baerthlein thought it was time to rectify Petruschky's description of morphology (polar flagella) and physiology (appearance of colonies on gelatin).

Pollak (1912) came to essentially the same conclusions as Baerthlein. He isolated eight organisms from stools of patients with enteric disturbances. All cultures were physiologically identical. Among the various characteristics given, indol was negative, milk not coagulated, litmus whey blue and no hemolysis of sheep blood. On original isolation the organisms showed considerable curvature. Cultivation on laboratory media resulted in a loss of curvature and there appeared straight rods with only a few curved individuals. Pollak concluded these forms were Bacillus faecalis alcaligenes. (The type of flagellation was not made clear.)

Castellani (1912) stated that Bacillus faecalis alcaligenes of the intestinal tract of man to be motile, gram negative, caused litmus milk to become alkaline, failed to produce indol and the Voges-Proskauer reaction, gelatin and coagulated serum were not liquefied. Glucose, mannite, maltose, dextrin, sorbite, galactose and laevulose media were turned acid.

Torrey and Rahe (1912-1913) confirmed the observations of Ferry (1910 and 1911) and M'Gowan (1911). There was marked uniformity of cultural reactions of several hundred strains of organisms isolated from animals with distemper. All were agglutinated by serum specific for a single strain, yet they separated into two groups in respect to their action on nitrates. The organisms were slowly motile in hanging drop preparations. Flagella were longer than the body of the bacillus and were five to six in number. (A photomicrograph showed very poorly stained flagella.) In the Smith fermentation tube with dextrose, growth was apparent in the open arm only. Although B. bronchisepticus was not an obligate aerobe it grew very poorly in the absence of oxygen. Plates incubated anaerobically showed only feeble growth. In gelatin stab growth appeared near the surface but was slight or absent in the lower part of the tube. There was no liquefaction of gelatin, no indol production, and pellicle formation. The cultural characteristics were in general the same as those given by both Ferry and M'Gowan. In litmus milk and on potato the reaction was similar to that of Bacillus faecalis alcaligenes. B. bronchisepticus exerts an active hemolytic action on dog, rabbit or guinea pig erythrocytes.

Smith (1913) studied Bacillus bronchisepticus and his description confirmed that of the earlier workers. He pointed out its biochemical

character approaches the pyocyaneus group. Like pyocyanea, Bacillus bronchisepticus is also a strict aerobe and without action on sugars. His strains of B. bronchisepticus, in a cursory comparison, were found to be culturally identical with pertussis. B. bronchisepticus antiserum agglutinated his pertussis strain to full titer. It was also noted that freshly isolated strains of the glanders bacillus and B. bronchisepticus are impossible to distinguish by the layer of growth on potato.

Furth (1913) reported a case which appeared clinically as typhoid fever. Bacterium faecalis alcaligenes was isolated from the blood and stool. The organism was described as a gram negative, motile rod (flagellation was not recorded) which did not liquefy gelatin, nor produce indol. It formed a blue colony on Conradi-Drigalski medium and a pellicle on broth. It was agglutinated by the patient's serum in a dilution of 1:120. The author noticed that his Hamburg stock strain was not agglutinated by antiserum to the organism isolated nor by the patient's serum.

Pacinotti (1913) isolated a variety of bacteria including paratyphoid bacilli and Bacillus faecalis alcaligenes from Italian vegetables. These vegetables were often fertilized by sewage and eaten raw. He points out that vegetables may be the source of infection by these organisms.

Kendall et al. (1913) observed a moderate amount of free ammonia produced in sugar free broth by Bacillus alcaligenes, however the typhoid and dysentery bacilli also produced similar amounts of ammonia in sugar free broth. There was evidence which seemed to indicate that in the presence of oxygen Bacillus alcaligenes can derive energy from glucose, or possibly from some unknown impurity as was shown by the increased growth in glucose media, and by

the slight increase of ammonia production in glucose broth compared to sugar-free broth. No measurable amount of acid or gas was produced from glucose. The organism was described as being negative in glucose and other listed carbohydrates. Gelatin and indol were negative. Motility was positive. Inasmuch as Bacillus alcaligenes did not produce acid in glucose, there was no sparing action of this sugar for protein as was manifested by the formation of as much or more ammonia in sugar broth as in sugar-free broth under the same conditions.

Kendall et al. (1913) observed the Shiga bacillus in a stool. Two days before the death of the patient a blood culture showed the following organisms: Shiga bacillus, paratyphoid B, Morgan's bacillus B, Alcaligenes and Streptococcus. (Cultural reactions were not described.)

Oppenheimer (1913) isolated Bacterium fecalis alcaligenes from a patient with pyelitis. (No further description of the organism was given.)

Kendall et al. (1914) found B. alcaligenes (description was not complete), to be an organism which fermented no sugars, did not peptonize nor coagulate milk but developed an increasing alkalinity in milk which was associated with the liberation of small amounts of ammonia. The ammonia amounted to less than 1% of the total nitrogen. The intensity of the reaction in milk corresponded closely with that obtained under similar conditions in broth cultures.

Shippen (1914) in studying the principal types of microorganisms in Baltimore milk isolated an organism which he said resembled B. faecalis alkaligenes of Petruschky (however it differed in regard to motility, morphology and gram reaction). It also resembled, according to Shippen, the organism

described by Conn (1899) as B. No. 66.

Straub and Kraus (1914) reported two cases of enteritis in which Bacillus faecalis alcaligenes was isolated. Case I organism was isolated from the blood. Blood agar colonies were pin head, blackish with hemolytic halo. The organism agglutinated in the convalescent serum of Case II in a 1:1000 dilution. The Case II organism was found in the urine. The patient's serum in a dilution of 1:1000 agglutinated the isolated organism. The organisms isolated in these 2 cases were considered variants of Bacillus faecalis alcaligenes even though the organism of Case I liquefied gelatin. The two organisms were compared with Schottmuller's Bacillus faecalis alcaligenes and are recorded in the table below:

	<u>B. faecalis alc.</u> <u>Schottmuller</u>	Case I organism	Case II organism
gram	negative	negative	negative
flagella	polar	polar	polar
motility	very motile	very motile	very motile
agar growth	good	thick white	thick white
gelatin	white slimy, no liquefaction	liquefaction	white, no liquefaction
bouillon	even turbidity pellicle	floccular pellicle	even turbidity
peptone H <sub>2</sub> O	pellicle	pellicle	pellicle
indol	negative	negative	negative
potato growth	brown	whitish, dry	gray-white, dry
litmus whey	blue	blue	blue
milk	clearing	clearing	clearing
dextrose agar	no gas	no gas	no gas
Drigalski's medium	blue	no growth	blue
Endo's medium	red	red, poor growth	reddish with slight inhibition

Rochaix and Marrotte (1914 and 1916) observed two patients with gastrointestinal symptoms from whom Bacillus faecalis alkaligenes was isolated from the blood. The two organisms were very similar. The patient's serum agglutinated typhoid bacilli in low titer, 1:50 and 1:100, but not the paratyphoids. Both patients had been vaccinated with typhoid bacilli. The organisms isolated were agglutinated by the respective patient's sera in high titer, 1:1200 and 1:1500. The organisms were similar to Straub's Case II and

Schottmuller's organism described by Straub. (Flagellation was not recorded.) The organisms cross agglutinated to titer but neither was agglutinated by antityphoid or antiparatyphoid sera.

Shearman and Moorhead (1916 and 1917) reported the isolation of Bacillus faecalis alcaligenes from the blood of 11 patients suspected of enteric infection. Agglutinins for the organisms isolated, in a dilution of 1-200 or higher, were found in the patient's serum. The organisms corresponded with the original description of Petruschky with one or two exceptions, a variation in motility, and in one strain there was peptonisation of litmus milk. The authors pointed out that the sugar reactions given by Castellani (1912) for Bacillus faecalis alcaligenes was in error.

Hirst (1917) isolated Bacillus faecalis alkaligenes in pure culture from the blood of 12 individuals. During 1916, 622 samples were examined bacteriologically and this organism was isolated 23 times out of 123 positive cultures (18.7%). Captain H. Wilshire, who was also in Alexandria at this time isolated this organism from the blood of three individuals. The table below is taken from Hirst's paper. It shows the principal characteristics of the organism as given by various workers. The nine strains of Hirst's Group I were pleomorphic, especially after repeated subculture on agar. All the strains of this group were nonmotile in primary culture. One became actively motile on subculture and several of the others became sluggishly motile. All the strains showed flagella when stained by the method of Nicolle and Morax. The typical arrangement of flagella was two at each end, but sometimes there were six flagella at the end. Indol was not produced, gelatin was not liquefied and milk was not peptonised. Alkali was produced in



	Petruschky	Klikaenko subgroup I	Straub & Krais strain I	Straub & Krais strain II	Rochaix & Marrotte	Castellani	Shearman	Hirst
motility	+	variable	++	++	++	+	variable	variable
flagella	peri	polar	polar	polar				polar
gelatin	-	-	+	-	-	-	-	-
bouillon	turbid	pellicle turbid deposit	flocc. pellicle	turbid pellicle	turbid pellicle	turbid	sl. turbid pellicle deposit	turbid pellicle deposit
carbohydrates	-	-	-	-	-	dext. +	-	-
litmus milk	blue	blue	blue	blue	blue	blue	blue	blue
milk		clearing	clearing	clearing	clearing		sometimes peptonized	no clear- ing
indol	-	-	-	-	-	-	-	-
pigment	potato brown	potato brown	none	none				potato brown
agglutination by patient's serum			1:1000	1:1000	1:1000 1:2000		1:200	1:50

glucose, lactose and sucrose broth. Hirst's second group of organisms, isolated from blood, were coccoid and no flagella were demonstrable. These strains were physiologically similar to the 9 strains in Group I. In Hirst's opinion the following characteristics served to identify Bacillus faecalis alkaligenes: Motility in hanging drop, gram stain, litmus milk, gelatin, appearance on agar slope, peptone bouillon appearance, lactose and glucose in litmus peptone water. The use of the agar slope avoids confusion with B. pyocyaneus and other pigment producers. The author observed that his isolates were fairly characteristic on MacConkey's lactose bile salt agar. The results in the table below strongly suggests that Bacillus faecalis alkaligenes multiplies in certain abnormal states of the bowel, and it is present in small numbers, if at all, in normal individuals.

	total	<u>Bac. faec. alkaligenes</u> found
enteric convalescents	43	16
dysentery convalescents	57	29
normal stools	50	nil

Thomson and Hirst (1918) reviewed the work of Shearman and Moorhead (1916 and 1917) as well as the report by Hirst (1917).

Castellani (1917) found in the Balkan and Adriatic zones cases of fever resembling enterica which may have been due to Bacillus faecalis alkaligenes. The author states the cultural reactions of Bacillus faecalis alkaligenes Petruschky to be as follows: Aerobic, nonspore-forming intestinal bacilli, motile, gram negative, gelatin negative, coagulated serum

negative, litmus milk alkaline, general turbidity in broth, indol not produced, Voges-Proskauer negative, and neither acid nor gas produced from glucose, lactose, sucrose, mannite, maltose, arabinose and many other listed carbohydrates. The typical organism produces strong alkalinity in all sugar broths, but certain strains are said to produce slight acidity in glucose and maltose. Some strains peptonize milk.

Strecker (1917) who wrote a dissertation at the University of Wurzburg concerning Bacterium alcaligenes, found they were curved rods similar to typical cholera vibrio. Often these curved rods formed spirills while at other times rods formed. Strecker studied the influence of media on the curved morphology but found no consistent correlation between the two. Using Zetnow's stain the organisms were bipolar lophotrichous with 1 to 6 flagella. Strecker was of the opinion that Petruschky was mistaken when he found peritrichous flagella, this mistake he thought was based on a culture contaminated with typhoid bacilli. Strecker examined many samples of urine and blood but never found Bacterium alcaligenes. The organism was very aerobic, as indicated by pellicle formation, growth in the upper layer of fermentation tubes, and growth in the upper layer of shake cultures. There was no liquefaction of gelatin and no acid or peptonization of milk. The colonies of the organism appeared similar to those of typhoid bacilli on Drigalski's medium. Litmus milk turned blue, no acid was produced from glucose, mannite, maltose or sucrose.

Evans (1918) described in detail three organisms, Bacterium abortus, Bacterium bronchisepticum and Bacterium melitensis in order that they might be compared. Carbohydrate, gelatin and indol reactions were negative.

B. bronchisepticum could be distinguished by its motility, abundant growth and intense alkaline reaction. It was concluded that a serological affinity existed among them. The serum of a cow hyperimmunized with Bacterium abortus was found to agglutinate melitensis and abortus to a titer of 1:1280 and Bacterium bronchisepticum to a titer of 1:40. The table below compares the three organisms.

	Litmus Milk	Urea	Aspara- gin	NO <sub>3</sub> reduced to NO <sub>2</sub>	Increase of pH
<u>bronchisepticum</u>	alk	+	+	-	2.0
<u>abortus</u>	alk	+	+	sl. or neg.	0.8
<u>melitensis</u>	alk	+	faint	sl. or neg.	0.8

Ferry and Noble (1918) found that after repeated subculture Bacterium pertussis had the same cultural reactions as Bacterium bronchisepticum. Bacterium bronchisepticum antiserum agglutinated not only Bacterium bronchisepticum but also Bacterium pertussis. Bacterium pertussis antiserum on the other hand agglutinated only the pertussis bacillus, with no effect on the strains of Bacterium bronchisepticum tested. Ferry and Klix (1918) confirmed, in most respects, the above serological relationships using complement fixation tests.

Kraus and Klaften (1918) isolated three strains of Bacterium faecalis alcaligenes from stools of patients with enteritis. They are compared in the table below. Gelatin, indol and sugars were negative. It was not clear if strain 1821 had one or many polar flagella. The author makes much of colony appearance and states growth on potato was atypical of Bacterium alcaligenes since growth was poor and no browning occurred.

strain number			
	1821	2502	2789
gram	-	-	-
motility	lively	lively	lively
flagella	polar	peritrichous	not demonstrated
aggl. with patient's serum	1:400	1:200	1:40
aggl. with B. faec. alc. serum	1:320	1:20	1:20
litmus whey	blue	blue	blue
milk	unchanged, alk.	unchanged, alk.	unchanged, alk.

Archibald (1918) isolated from the blood of a native of Sudan with atypical enterica an organism which he named B. vivax. It was described as a gram negative, actively motile bacillus 1.6 microns long. (The flagellation was not recorded.) A pellicle was formed in broth. Gelatin was not liquefied. Litmus milk was not acidified but became alkaline in 48 hours. Indol and Voges-Proskauer reactions were negative. Nitrates were reduced. Acid, but not gas, was formed in galactose and mannitol, while an alkaline reaction without gas formation occurred in glucose and other listed carbohydrates. The patient's serum, in dilutions of 1:30 and 1:60, completely agglutinated the bacillus isolated.

Webster (1919) recovered Bacillus faecalis alkaligenes from blood of 3 infants with enteric fever. The organisms were reported as being gram negative and motile with alkali production in sugars and milk. (Flagella studies were not recorded.)

Castellani and Chalmers (1919) defined the genus Alcaligenes as

follows: Gram negative asporogenous cylindrical long or short cells with capsules and which divide in one direction only. Growth good in ordinary laboratory media, gelatin not liquefied, aerobes often facultative anaerobes, no fluorescens or pigment production. Do not ferment glucose or lactose and are characterized by their general lack of fermentative power and by actually increasing the alkalinity of the media. Milk is not clotted or rendered alkaline. Alcaligenes faecalis (Petruschky, 1896), emendavit Castellani and Chalmers, 1918\*. Here also is listed Alcaligenes vivax (Archibald, 1918) which was obtained from the blood of an individual with intestinal disorder in the Anglo-Egyptian Sudan. The organism produced acidity in galactose and mannitol and was characterized by its marked motility. B. faecalis alkaligenes (Petruschky) gave the following negative reactions: Gram, gelatin, coagulated serum, lactose, saccharose, dulcitate, mannite, glucose, maltose, dextrin, raffinose, arabinose, adonite, inulin, sorbite, galactose, levulose, inosite, salicin, amygdalin, isodulcitate, erythrite, glycerine, indol and Voges-Proskauer. In broth it produced a general turbidity. Motility was positive and litmus milk alkaline. The typical B. faecalis alkaligenes produces a strong alkaline reaction in all sugar broths but certain strains are said to produce slight acidity in glucose and maltose. Some strains peptonize milk.

Castellani and Chalmers (1919) use the spelling Alcaligenes and Alkaligenes, their new genus designation, indiscriminately. The name

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\*The citation for 1918 is probably in error, as no description of the genus can be found as of this date, hence it should read 1919. This is in agreement with Weldin (1927).

originally assigned by Petruschky to represent the beer bacillus was also given a variant spelling by these taxonomists.

Rochaix and Blanchard (1920) tabulated cases of Bacillus faecalis alcaligenes infection and found it most common in spring and summer. They concluded that both warm climates and warm seasons are factors affecting the incidence.

Watson (1921) reported the isolation of Bacillus faecalis alcaligenes from the blood of four soldiers in India. The patient's sera showed high agglutination titers with the typhoid organism and low agglutination titers with Bacillus faecalis alcaligenes. (A description of the organism did not accompany the report.)

de Magalhaes and Bettencourt (1921) isolated Bacillus faecalis alcaligenes from the blood of a patient using a bile medium. The organism was gram negative, no spores, very motile with peritrichous flagella. No acid from lactose or glucose peptone broth. Milk was not coagulated but became transparent and yellowish (alkalinization). Litmus whey was unchanged. Indol was not produced and gelatin was not liquefied. The patient's convalescent serum agglutinated the organism isolated in a dilution of 1:400.

Ravenel (1921) isolated Bacillus faecalis-alkaligenes from the spleen and intestinal patches of a cadaver. The patient had been ill with symptoms of typhoid fever. Dextrose and other carbohydrates listed were not fermented. Litmus milk remained unchanged in appearance for 8-10 days, then it became darker and showed a slight gelatinous coagulation. (The description is not complete as to gram reaction, motility and flagellation.) The organism was considered the causative agent in the production of the

intestinal lesions.

Braun and Cahn-Bronner (1921) reported that Alcaligenes faecalis would grow aerobically in a simple medium with ammonium lactate as a source of nitrogen and carbon. Growth was poor but improved by addition of sulfate ion and aeration. Sodium chloride or potassium chloride had to be present for growth. (A description of the organism was not included.)

Cooke (1921) reported Bacillus ammoniagenes as the etiologic agent of ammoniacal excoriation of the buttocks of infants. The organism was an asporogenous gram positive bacillus which did not ferment sugars. This yellowish pigmented organism hydrolyzed urea with the production of ammonia. (This organism was named Alcaligenes ammoniagenes in Bergey's Manual of Determinative Bacteriology, 3rd edition 1930, page 367. See the report reviewed in this paper by Cooke (1926).)

Stitzer (1922) isolated from water and stools 14 vibrios which were not agglutinated by anticholera serum. They were recognized as Bacillus faecalis alcaligenes by their biochemical nature. He also studied the intestinal flora of cholera patients. On the second day of illness and through to the end of the disease when the stool became formed, Bacillus faecalis alcaligenes was predominantly found. (A description of the organism was not given.)

Mudd and Warren (1923) isolated Vibrio percolans (new species) from hay infusion. This gram negative, asporogenous organism was comma shaped to straight, actively motile with one polar flagellum, though frequently 2 or 3 flagella were present. Growth in peptone broth had a pellicle, litmus milk unchanged, no liquefaction of gelatin, no reduction of nitrates, no indol



production, no fermentation of dextrose, lactose, sucrose and other listed carbohydrates.

Khaled (1923) in Egypt noted the blood culture from an enteric type infection resulted in the isolation of B. faecalis alcaligenes. (A description of the organism was not recorded.)

Weldin and Levine (1923) published a key to the species and varieties of the colon-typhoid group of organisms. Under the genus Bacterium was listed the sub-genus Alcaligenes described as gram negative rods growing well on artificial media, asporogenous, nonmotile or motile with peritrichous flagella. Neither acid nor gas is produced from glucose.

Bergey et al. (1923) defines Alcaligenes Castellani and Chalmers as motile or nonmotile rods, generally occurring in the intestinal canal of normal animals. Do not form acetyl-methyl-carbinol. Do not ferment any of the carbohydrates. The type species, Alcaligenes fecalis (Petruschky) Castellani and Chalmers, was described as follows:

Rods: 0.5 by 1.0 to 2.0 microns, motile by means of peritrichous flagella.

Gram negative.

Gelatin stab: Gray surface growth. No liquefaction.

Agar slant: White, glistening.

Broth: Turbid, with thin pellicle.

Litmus milk: Alkaline.

Potato: Scanty to abundant, yellowish to brownish.

Indol not formed.

Nitrates reduced to nitrites.

Does not form acid or gas in carbohydrate media.

Aerobic, facultative.

Habitat: Intestinal canal. Found in endocarditis but generally considered nonpathogenic.

Alcaligines bronchisepticus (McGowan) was also included in this genus:

Rods: 0.4 to 0.5 by 2.0 microns, motile, possessing peritrichous flagella.

Gram negative.

Gelatin stab: No liquefaction.

Agar slant: Lustrous and moist.

Broth: Distinctly turbid, with thin, gray pellicle and ropy sediment.

Litmus milk: Alkaline.

Potato: Fairly abundant, brownish, glistening.

Indol not formed.

Nitrates not reduced.

No acid or gas in carbohydrate media.

Ammonia formed from urea and asparagin.

Aerobic.

Habitat: Believed to be the cause of distemper in dogs.

Kollath and Lubinski (1924) reported the isolation of a gram negative motile rod with curved shape from a patient with diarrhea. The organism was isolated from the stool and at autopsy from the intestine, liver, spleen and gall bladder. Flagella stain (Peppler) showed mainly polar monotrichous, rarely two flagella, no amphitrichous or peritrichous types were found. (Photomicrographs were not published.) In media with dextrose, lactose, sucrose, maltose or mannite the litmus turned blue. Colonies on rabbit blood agar were hemolytic. In the course of study the original curved

form of the organism largely disappeared and the organism became straight. Anticholera serum did not agglutinate the organism nor a stock culture of Alcaligenes.

Brown (1926) reported a case clinically like whooping cough caused by Brucella bronchiseptica. The organism was actively motile but the flagellar morphology was not recorded.

Row (1926) reported the isolation of Bacillus faecalis alkaligenes from the stool of a patient whose general condition was that of typhoid fever. (A description of the isolated organism was not given.)

McDonald and Levine (1926) observed, in a synthetic medium containing asparagine and ammonium lactate as nitrogen and carbon sources, uric acid to be produced by B. faecalis alkaligenes. Uric acid was also produced in a peptone medium. In the purine-free medium B. pyocyaneus always failed to produce uric acid, while on peptone medium it produced uric acid freely.

Levine and Soppeland (1926) in their study of bacteria in creamery wastes isolated Bacterium alcaligenes (Petruschky) Lehmann and Neumann. The morphology was described as rods, no spores, motile and gram negative. (The flagellar morphology was not given.)

Dudgeon (1926) reported a study of the human intestinal flora under normal and abnormal conditions. The author observed that B. faecalis alkaligenes was described as a normal inhabitant of the intestinal tract. However, from his own experience this bacillus was seldom isolated from feces on brom-thymol blue lactose agar.

Thompson (1927) observed Alcaligenes faecalis in a medium containing 0.2% dextrose as carbon source and 0.2% glutamic acid as nitrogen source.

(A description of the organism was not included in the report.)

Cooke (1926) and Cooke and Keith (1927) described more fully the organism which she originally named Bacillus ammoniagenes but now suggests Bacterium ammoniagenes. It was described as a rod, nonmotile, asporogenous, gram positive, litmus milk unchanged except for production of slight alkalinity, gelatin not liquefied, and sugars not fermented. Cora B. Gunther, in a communication to Einar Leifson dated 17-VII-51, stated Bacterium ammoniagenes #6871 American Type Culture Collection and a strain she had received directly from J. V. Cooke as Bacterium ammoniagenes to be gram positive. This is in agreement with the original description. H. J. Conn, who revised the genus Alcaligenes in Bergey's Manual of Determinative Bacteriology, 5th edition (1939) continued to list the organism as Alcaligenes ammoniagenes and as gram negative. The 6th edition of the Manual of Determinative Bacteriology (1948) lists this organism in the Appendix to the genus Alcaligenes.

Wyatt (1927) isolated Bacillus fecalis alcaligenes from the blood and stool of a nurse with mild typhoid-like symptoms. The serum agglutinated the fecal strain to a titer of 1:80. (A description of the organism was not given.)

Weldin (1927) suggested the generic name Alkaligenes, used by Castellani and Chalmers (1919), a variant spelling of Alcaligenes should no longer be recognized. Weldin opposed Evans' suggestion that a distinction be made between Alcaligenes and Brucella on grounds of pathogenicity, hence he included the melitensis-abortus group in the genus Alcaligenes. The genus included only those organisms which produced neither acid nor gas from

glucose.

Lehmann and Neumann (1927) named the organism under consideration Vibrio alcaligenes (Petruschky) L. and N. This was synonymous with Bacillus faecalis alcaligenes Petruschky and Bacterium alcaligenes in their previous editions. The authors were convinced by the investigation of Strecker (1917) that this organism should be designated as Vibrio or Spirillum and was unrelated to the colon-typhoid group. The organisms were described as curved rods, often distinct vibrios and spirillum forms with 1 to 6 polar flagella. There was no liquefaction of gelatin, growth on potato was luxuriant and brownish, on sugar-free media it did not form more alkali than most bacteria. It did not produce acid from sugars, and even dextrose broth became strongly alkaline. The authors recognized that B. mariense Klimenko (1908) and Vibrio terrigenus Gunther (1894) were somehow related to these organisms.

Gray and Thornton (1928) described two new species of soil bacteria.

Pseudomonas dacunhae n. sp.

Rods: 3 by 0.5 to 0.8 microns, curved, motile. (According to the drawings 1 to 6 polar lophotrichous flagella.)

Gelatin stab: Nailhead.

Agar slant: Pale buff, smooth, shining growth.

Broth: Cloudy.

Nitrates not reduced to nitrites.

No acid produced from carbohydrates.

No diastase produced.

Phenol was attacked.

(Bergey's Manual of Determinative Bacteriology, 6th edition (1948) p. 105, retains this organism in the genus Pseudomonas and states it reduces nitrates to nitrites.)

Vibrio cuneata n. sp.

Rods: 1 to 3 by 1 microns, curved in a short spiral, motile. (According to the drawings they have 1 to 3 polar multitrichous flagella.)

Gram negative.

Gelatin stab: Liquefied.

Agar slant: Whitish, smooth, shining.

Nitrates not reduced to nitrites.

No acid produced from carbohydrates.

No diastase produced.

Naphthalene was attacked.

(Bergey's Manual of Determinative Bacteriology, 6th edition (1948) p. 199, retains this organism in the genus Vibrio.)

Takayanagi (1928) isolated 58 strains of Bacillus faecalis alcaligenes from feces and urine of typhoid patients, and from pus in appendicitis. These gram negative organisms were poorly flagellated, peritrichous, often only 2 flagella appeared at both ends (the meaning of this description is not clear.) Dextrose and other sugars were not utilized, milk was not changed, alkali formed in litmus whey, potato growth was moist and brown. Antigenically the 58 strains were placed in 15 groups showing little crossing.

Monias (1928) found that the organisms named Bacterium faecalis alcaligenes, isolated by various authors, could be separated morphologically and physiologically into two groups. The organisms of the first group, which included Petruschky's beer bacillus, were motile with peritrichous flagella and fermented carbohydrates. The second group with polar flagella corresponded to the group Pseudomonas, Migula. He was of the opinion these species could be called Pseudomonas alcaligenes. The 10 organisms in this second group were gram negative, some straight and some curved with polar flagella, milk became alkaline and no acid was produced in carbohydrates.

Gondolf and Stringer (1928) found 10 cases of B. alcaligenes urinary tract infections.

Marshall (1928), Daniel and Green (1929) and Danbolt (1929) reported

the isolation of Bacillus faecalis alkaligenes from stools, blood and gall stones. (The flagellar morphology was not recorded.)

Weiss (1929) suggested the name Alcaligenes appendicalis for the organism he isolated from the blood in three cases of acute appendicitis. It was described as a gram negative rod; nonacid-fast; nonmotile without flagella; no spores, capsules or metachromatic granules. There was no pellicle in broth, no liquefaction of gelatin, litmus milk unchanged, potato showed only scant growth without pigment. Blood agar colonies were grey-white with no hemolysis. Hydrogen sulfide was not detected in lead acetate agar. None of the commonly used laboratory sugars were fermented. There was no indol produced, nitrates were reduced, methyl red and Voges-Proskauer reactions were negative. Hyperimmune serum did not agglutinate B. typhosus. It agglutinated the homologous strain 1:2000, Alcaligenes fecalis 1:30, Alcaligenes bronchisepticus 1:30, Alcaligenes abortus 1:120, Alcaligenes melitensis 1:120, and Alcaligenes bookeri 1:300. The descriptions of species of Alcaligenes in Bergey's Manual (1925) did not fit this organism, hence the new name was suggested.

Kendall and Ishikawa (1929) observed that the reduction of methylene blue by "resting cells" in the presence of certain carbon compounds was precisely paralleled by the fermentation of these same compounds in culture media inoculated with identical strains of proliferating bacteria. The organisms were not described but Bacillus alkaligenes and Vibrio H/61 did not ferment any sugars. These two organisms did not reduce methylene blue in the presence of a large number of sugars but there was reduction with other compounds. Some of the results are listed in the table below.

	glycerol	formic acid	succinic acid
<u>B. alcaligenes</u>	-	+	+
<u>Vibrio</u> H/61	+	+	+

Topley and Wilson (1929) state that Brucella bronchiseptica produces a hemolysin acting on rabbit red blood cells, no production of hydrogen sulfide, grows best under aerobic conditions, no growth under strictly anaerobic conditions. It is motile by means of 4 to 6 peritrichous flagella.

Gracey (1930) and Lindemann (1930) reported several isolations of Bacillus faecalis alcaligenes from blood, gall bladder, urine and testicular abscess. (The flagellar morphology of the organism was not reported.)

Ryti (1930) isolated 6 strains of Bacillus faecalis alcaligenes from blood, feces and stomach contents. All six were gram negative, motile rods, no spores, no capsules, 6 to 20 peritrichous flagella. Indol was negative, gelatin negative, alkaline reaction in glucose, lactose, mannite, maltose, salicin and sucrose broths, no hemolysis of human blood, milk not coagulated, potato moist, slimy, yellow-brown layer. The author felt these organisms should retain Petruschky's designation. He was of the opinion that the polar flagellated, alkali producing rods should be another group closely related to Bacterium fluorescens non-liquefaciens. The lophotrichous group of Lehmann and Neumann and Pseudomonas of Migula forms one group which might be called Pseudomonas alcaligenes. Strecker's polar flagellated organisms, named Vibrio alcaligenes by Lehmann and Neumann, should not be confused with the peritrichous Bacillus faecalis alcaligenes.

Castellani (1930 and 1930) reported the isolation of Alkaligenes



alkalofaecidis from tonsillar crypts. This bacillus was motile, gram negative, no acid produced from carbohydrates, gelatin and coagulated serum were not liquefied, no indol was produced, alkali was produced in litmus milk.

Barrow (1931) and Gatewood (1931) isolated Bacillus faecalis alkaligenes from patients with conjunctivitis and meningitis, however a description of the isolated organism was not given.

Evans (1931) described an organism she named Alcaligenes faecalis subspecies radicans. The organism was obtained from the blood of a patient with fever resembling typhoid. It was described as being gram negative; asporogenous; motile by means of peritrichous flagella; no capsule; wrinkled, dull, moderate amount of growth appeared on an agar slope in 24 hours; no pigment; liquefaction of gelatin began from the surface. (Photomicrographs showing flagella were not published.) Litmus milk growth was accompanied by the development of an alkaline reaction which increased for a week or more. No growth appeared on potato and there was no hemolysis of red blood cells. The organism was aerobic. An alkaline reaction was produced in broth containing dextrose, lactose, sucrose, maltose, mannose, xylose, arabinose and other carbohydrates. Growth did not occur in synthetic media containing inorganic salts and cystine, tryptophane or uric acid as a source of nitrogen. No growth in Koser's citrate medium. Acetyl-methyl-carbinol, hydrogen sulfide and indol were not produced. Antiradicans serum did not agglutinate Alcaligenes faecalis or other enteric organisms. The author stated the organism isolated by Straub and Kraus (1914) from blood holds an intermediate position between the species faecalis and the subspecies radicans.

Sloboziano and Nasto (1931) obtained Bacillus faecalis alkaligenes

from abdominal paracentesis fluid of twins with fatal peritonitis. The organism was characterized as being motile; gram negative; abundant, dull gray viscous growth on agar; gelatin not liquefied; broth turbid with a pellicle; indol not produced; litmus milk became blue; mannitol, maltose, saccharose, lactose, galactose, and arabinose not fermented. (No record of flagellation.)

Asbelow and Mostowa (1931) isolated 48 strains of Bacillus faecalis alcaligenes from the blood of patients suspected of having typhoid fever. They were gram negative rods, often curved and with lively motility, colonies on Endo's medium appeared like those of typhoid bacilli, indol and gelatin were negative, milk was not coagulated, litmus milk turned blue, no acid or gas in various carbohydrate media. Sixteen samples of normal blood serum agglutinated about 20% of the strains. Sera prepared in rabbits against 6 strains agglutinated all the strains to 1:1000 or over with the majority agglutinating to titer. Nine strains of similar organisms isolated from water were serologically heterogenous and showed no antigenic relationship to the blood strains. (No mention of flagellation was made.)

Anderson (1933) cultured the blood from two patients exhibiting a syndrome simulating rheumatic polyarthrititis and isolated Bacillus alcaligenes which was gram negative and left carbohydrate media unchanged. (The organism was not described further.)

Mason (1934) isolated Bacillus fecalis alcaligenes from spinal fluid following spontaneous opening of a meningocele. The bacillus was gram negative, motile, produced no acid or gas in carbohydrate media, indol negative, litmus milk became alkaline and there was no liquefaction of gelatin.

There was no agglutination of the organism by the patients serum. (The flagellar morphology was not recorded.)

Stuart et al. (1934) isolated Bacillus alkaligenes faecalis from the urine and a renal calculus removed from the kidney of a case of chronic lithiasis. The organism was not described other than that it was urea negative.

Spray and Hawk (1934) reported a case of meningitis secondary to bilateral otitis media. The patient died and Alcaligenes fecalis was isolated from the spinal fluid. The actively motile organism was a short, gram negative rod showing bipolar staining, no spores or capsules, gelatin was liquefied, broth had a diffuse turbidity with a thin pellicle, litmus milk had no initial acidity and became strongly alkaline, carbohydrate broth became strongly alkaline to brom-cresol-purple, indol was not produced, nitrates were not reduced to nitrites, blackening did not appear on lead acetate peptone iron agar and colonies on Endo's medium were colorless. The organism was considered as the specific infecting agent responsible for the death of the patient.

Leifson (1935) reported new culture media based on sodium desoxycholate. He observed that Bronchisepticum (peritrichous) grew fairly readily and Alcaligenes (lophotrichous) grew well (one strain) on desoxycholate agar. Desoxycholate citrate agar generally inhibited Alcaligenes almost completely but some strains developed tiny colonies in 24 hours. Various types of monoflagellated bacilli including pyocyaneus grew well on the medium. The author stated that only polar flagellated bacilli were being included in the genus Alcaligenes.

Saisawa and Takahashi (1935) recognized that Bordet and many workers long since described the bacteria causing whooping cough as being without flagella. These workers constantly found the Bordet bacillus to be flagellated. The flagellation was the same as that seen on Brucella bronchiseptica.

Nyberg (1935) examined 134 organisms labeled B. alcaligenes, 82 of which were divided into 2 distinct groups.

Group A: 71 strains

32 sewage

6 feces of normal individuals

22 feces of typhoid and paratyphoid patients

5 urine-pyelitis

6 old laboratory strains received from Ryti

They were short thick coccoid bacilli easily mistaken for Staphylococci. No spores were formed. They were mostly nonmotile but in hanging drop some individuals moved. Zettnow's stain showed they were peritrichous with 1 to 4 flagella seldom 6 to 7. No liquefaction or change in milk; indol, methyl red, Voges-Proskauer tests were negative; no acid from sugars, however bouillon sugar medium changed from pH 7.45 to 7.35 in one series, in another series from 7.21 to 7.0. When the pH rose from 7.45 to 7.75 the organism did not belong to this group. Occasionally lophotrichous and transitional forms were encountered which lowered the pH. Mutants and variants were isolated from this group which peptonized milk and gave acidity in xylose and dextrose. This description does not correspond to Petruschky's original description, but Nyberg considered them Bacterium faecalis alcaligenes, in spite of the poor motility and increased acidity of sugar media.

Group B: 11 strains

8 typhoid-paratyphoid feces which also contained Group A

3 sewage

~~Long slender slightly curved rods with lively motility, slimy and spreading~~

growth. Lophotrichous with 3 to 4 flagella. These were identical with Vibrio alcaligenes Lehmann and Neumann. This organism had been called Alcaligenes faecalis in the literature but the author called them Vibrio alcaligenes. They produced an alkalization of the dextrose medium.

Group C: 52 strains  
 2 typhoid-paratyphoid feces  
 50 sewage, incompletely investigated

They have characteristics of both Group A and B. Motile rods which change the pH of sugar media from 7.45 to 6.3 or 6.6. (Notice that this is lower than Group A.) They have about 4 terminal flagella, but behave like Group A on many media. These were referred to as Pseudomonas forms.

Hasen and Mortillaro (1936) described an organism isolated from the blood in a case with typhoid fever symptoms. The organism was referred to as a hitherto undescribed microorganism of the Alcaligenes group and was described as follows: Small gram negative aerobic bacilli with no flagella, capsules and polar bodies were not demonstrable. No hemolysis on horse blood infusion agar. No liquefaction of gelatin. Growth moderate and of light cream color on potato. Litmus milk was unchanged. In milk containing either phenol red or brom-cresol-purple, a slight alkaline reaction was observed. Indol was not formed. Acetyl-methyl-carbinol was not demonstrated and the methyl red test was negative. Carbohydrates in peptone water medium were not fermented, however a strong alkaline reaction was observed. There was no serological relationship of the organism to Brucella bronchiseptica, abortus or melitensis.

McIntyre (1936) reported the isolation of Fecalis alcaligenes from the blood of a patient with acute hepatitis and jaundice. The organism was

reported as being motile but was not described further.

Henriksen (1937) reported, in a dissertation on the bacterial flora of the respiratory tract, the isolation of a nonmotile, hemolytic organism he named Alcaligenes hemolysans. It was isolated from eleven specimens of sputum of patients with chronic bronchitis, asthmatic bronchitis and other conditions. This organism was very strongly hemolytic and the hemolysin was filtrable and antigenic. The strains grew vigorously in broth, forming a pellicle and a somewhat viscous deposit. Some strains also grew profusely below the surface with the medium becoming densely turbid. Milk slowly formed a soft coagulum which later peptonized, but remained alkaline. Gelatin was liquefied and coagulated serum was not liquefied. Nitrates were not reduced. Fermentation of dextrose, lactose, sucrose, maltose and mannitol did not occur. All strains were uniform biochemically and serologically.

Bradford and Slavin (1937) reported that the morphological, biochemical and immunological characteristics of Haemophilus pertussis, Brucella bronchiseptica and the parapertussis bacillus were similar.

Eldering and Kendrick (1937 and 1938) observed the close relationship of Bacillus pertussis and Bacillus bronchisepticus to Bacillus parapertussis on the basis of morphology, growth on various media, biological characteristics, serological reactions including agglutinin absorption tests and certain reactions in animals. Although these organisms were similar parapertussis was identical with neither of the other two. They point out that Bacillus parapertussis can not be placed in the genus Haemophilus since it grows readily on ordinary blood free media.

Goldberg (1938) presented a case which clinically appeared as

meningitis. The blood culture on 2 occasions resulted in the isolation of what he called Bacillus alcaligenes. It was gram negative, caused litmus milk to become alkaline and Russel's medium to show no gas but slight acid was present with an alkaline slant. (No further description was given.)

Watanabe (1938) believed that Bacillus pertussis changes into a motile animal type Bacillus pertussis. These two organisms resemble B. bronchisepticus in morphology, cultural behavior, antigenicity and biochemical characteristics as well as in the toxicity and intracutaneous reactions of the toxic substance obtained from the bacterial water extract. The author was of the opinion that the change from Bacillus pertussis to the motile animal type Bacillus pertussis was a mutation and not a variation, since he had observed a human case naturally infected with the animal type and the motility was not reversible. The author also was of the opinion that the reason Europeans and Americans had not recognized the experimental whooping cough of dogs was that this bacillus was not regarded as a variant of the Bordet bacillus, but mistaken for B. bronchisepticus.

Berthelot and Armoureux (1938) reported the isolation of Bacterium alcaligenes from purulent knee exudate.

Björ and Hartmann (1938) reported the isolation of Bacterium alcaligenes from purulent knee exudate.

Evans and Maitland (1939) described the toxin of Brucella bronchiseptica and found it similar to the toxin of Haemophilus pertussis. Neither toxin was antigenic and both were heat labile. They also found that sera from cases of whooping cough failed to neutralize pertussis toxin. The relationship of these organisms was evident since they produced the same kind

of acute lesions in the lungs of experimentally infected mice. Antigenic relationship was demonstrated by immunization, precipitation, complement fixation, agglutination and agglutination absorption tests. The cultural and morphological similarities were also described.

Bruckner and Evans (1939) studied the toxin of Haemophilus pertussis, Brucella bronchiseptica and Bacillus paraptussis. All three produced similar lesions in experimental animals. Their toxins were separable from the bacterial antigens and were alike in their reactions to heat, formalin, filtration and lack of antigenicity. The results of agglutination, complement fixation and precipitation tests are in agreement with those of Eldering and Kendrick and show that the three organisms are closely related antigenically. The authors concluded that the data justify placing the three organisms in the same genus.

Conn et al. (1940) state that the original description of Alcaligenes faecalis (Bacillus faecalis alcaligenes Petruschky) makes it clear that it is a peritrichous, non-spore-forming rod of intestinal origin, turning milk alkaline and produces no acid in sugar broths. Conn recognized that many workers described it as polar flagellated. None of the above characteristics, Conn points out, serves to distinguish it from Rhizobium, Phytomonas, Bacterium radiobacter, Chromobacterium and Achromobacter. Conn collected 22 cultures labeled Alcaligenes faecalis. All agreed with Petruschky's original description of the organism except for three strains, one of which was nonmotile the other two were polar flagellated. The other 19 had what the author called "degenerate peritrichous flagellation." All but a small minority of the cultures had the following characteristics. They produced



alkalinity without peptonization in litmus milk; grew poorly in synthetic glucose media but growth was greatly stimulated by the addition of a small amount of yeast extract; there was no evidence of any acid from sugar except  $\text{CO}_2$ , but some of the cultures produced this in abundance in a glucose medium suitable to their growth. About half the cultures studied produced nitrites from nitrates. He concluded there was a well recognized organism to which the name Alcaligenes faecalis could be assigned. Conn, a soil bacteriologist, cautioned that all intestinal organisms, producing alkalinity in litmus milk and no acid in glucose, may not be Alcaligenes faecalis since other types of organisms also have these characteristics. Organisms isolated from fermenting sauerkraut and from a Cuban sugar mill proved impossible to distinguish from the typical Alcaligenes faecalis. Many soil bacteria similar in physiology were found to have the same type of degenerate flagellation as Alcaligenes faecalis. (Photomicrographs did not accompany the paper but drawings were included to represent the types of flagellation of the various organisms.)

Barron and Friedemann (1941) observed that Alcaligenes faecalis did not oxidize glucose but did oxidize hexose monophosphate and hexose diphosphate. This failure to oxidize glucose was stated to be due to inability to phosphorylate. (The organism was not described.)

Pohl and Raymond (1941) described a case with abscess on the neck in which the etiologic agent was believed to be Bacillus faecalis alcaligenes. The differentiation of this organism from Shigella alkalescens and Bac. alkalescens was said to be indeterminate. (The bacteriological description of the isolated organism was not included in the report.)

Eldering (1941 and 1942) reported the isolation of a protein free polysaccharide from Brucella bronchiseptica, Haemophilus pertussis and the parapertussis bacillus. Protection tests with the polysaccharide fraction and whole killed organisms emphasized the close relationship of these three organisms.

Voorhies and Wilen (1942) recorded a case of bacteremia by Alcaligenes faecalis. The bacteremia and fever lasted four months. Interesting manifestations were a polyarthrititis and meningitis. The organism was characterized as slightly liquefying gelatin, no fermentation of listed carbohydrates. Litmus milk became alkaline in 4 days. The organism was a slightly motile gram negative rod. No agglutination occurred with the patients serum and the isolated organism.

Ahad (1942) observed Bacillus faecalis alcaligenes in catheterized urine of a patient with cystitis. It grew out on MacConkey's agar and was a short, motile, non-spore-bearing rod. Glucose and other carbohydrates listed were not fermented but alkali was formed. Litmus milk showed progressive alkali formation. Voges-Proskauer, indol and methyl red tests were negative. Liquefaction of gelatin or reduction of nitrates did not occur. Convalescent serum agglutinated the isolated organism in a dilution of 1:200.

Conn (1942) attempted to learn whether certain soil organisms belonged to the genus Alcaligenes. When the type species Alcaligenes faecalis Castellani and Chalmers (Bacillus faecalis alcaligenes Petruschky) was investigated, it was found that the type species was not well characterized and, the genus was ill-defined. The study showed that at least three species were being distributed as Alcaligenes faecalis: 1) a peritrichous rod

requiring organic nitrogen, 2) a lophotrichous rod requiring organic nitrogen, and 3) a peritrichous rod using nitrate or ammonium salts as sole sources of nitrogen. It seemed probable that the first was the organism originally described by Petruschky. This organism appeared very similar to Bacterium bronchisepticum. The second was excluded from consideration as the Petruschky bacillus because it had lophotrichous flagella. The third agreed with Petruschky's description and had been distributed by type culture collections. Its fecal origin was doubtful and since it can grow on synthetic media it probably belongs to a different genus. He concluded that strains recovered from soil which did not produce acid or gas from carbohydrate media were related to the third and not to the first group.

Conn (1942) reviewed the classification of Petruschky's organism through several editions of Bergey's Manual of Determinative Bacteriology. Of the various cultures which he accumulated from the American Type Culture Collection, the National Collection of Type Cultures, C. Nyberg in Helsinki, Pribram's collection in Chicago, E. G. D. Murray in Montreal and others, some were lophotrichous and obviously belong elsewhere in the taxonomic scheme. He recognized that bacteriologists generally were regarding any nonfermenting gram negative rod of fecal origin as Alcaligenes faecalis. In comparing the soil forms with the intestinal forms he found a similar morphology, and acid and gas tests on ordinary media were always negative. Production of nitrite from nitrate proved a variable character. Two features were said to be useful for making distinctions: 1) ability to grow on synthetic media, 2) production of  $\text{CO}_2$  from glucose. The following two media were used:

## Medium I

$(\text{NH}_4)_2\text{HPO}_4$	1.0 g.
KCl	.2
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	.2
yeast extract	.2
agar	15.0
water	1000.0

## Medium II

Same as above with the addition of glucose.

The soil forms showed scanty growth on Medium I, but very vigorous growth on Medium II. Only five of the cultures received as Alcaligenes faecalis behaved similarly. The remaining strains of Alcaligenes faecalis showed poor or no growth in either medium. There was no evidence that Alcaligenes faecalis could grow without organic nitrogen. Conn concluded that abundant  $\text{CO}_2$  was produced by the soil forms but not by typical Alcaligenes faecalis. Based on this evidence Conn questions if anyone knows just what constitutes an Alcaligenes, since it could be either those organisms which grow on his synthetic media or those which require organic nitrogen. Both types were being distributed under the same name.

Pirot and Bourgain (1942) reported aberrant orchitis in guinea pigs as being due to Bacillus alkaligenes faecalis var. maritense.

Leifson's textbook (1942, page 178) shows distinctly, for the first time, the peritrichous flagellation of Brucella bronchiseptica. He also suggests that this organism may be out of place in the genus Brucella and should, perhaps, more properly be placed in the genus Alcaligenes. The

inconsistency regarding the basic characteristics of the genus Alkaligenes was recognized (page 181). The Alkaligenes most commonly encountered in stools have very characteristic polar flagella, (photomicrographs shows lophotrichous forms) quite different from the flagella of most bacteria. The peritrichous type was stated to be inhibited to a great extent by bile salts. Anatomically and physiologically the latter seemed identical with Brucella bronchiseptica.

Weintraub and Neter (1943) reported a case of bacteremia complicating diabetes mellitus in which Bacillus alcaligenes was isolated 3 times from the blood. No specific agglutinins were found during the illness. Growth of the organism on plain agar was abundant. Dextrose and other listed carbohydrates were not turned acid. Indol was not produced and gelatin was not liquefied. Conn examined the strain and stated it required organic nitrogen to grow. (Morphological studies were not recorded.)

Raeburn (1944) described 3 cases of enteritis occurring in Egypt which appeared to be due to an organism like Nyberg's Bacterium alkaligenes. Serum of the patients agglutinated the isolated organism in 1:10 or 1:25 dilution. The organism isolated was a gram negative rod, feebly motile. Dextrose and other listed carbohydrates were not fermented. Indol was not produced and litmus milk became slightly alkaline. In the litmus milk reaction the organism differed from Nyberg's Bacterium alkaligenes. The author commented that Nyberg's proposed separation provided no clue as to which, if either, of his groups of organisms were responsible for the infections recorded in the literature.

Chauduri (1944) isolated Bacterium alcaligenes and Bacterium

metacaligenes from 6 patients, some of which had specific agglutinins in the serum. (There was no description of the isolated organism.)

Shulman and Johnson (1944) reported the recovery of a microorganism from blood and spinal fluid of a premature infant. It was a short, straight, gram negative bacillus. Flagella, endospores, capsules and metachromatic granules were absent. There was no hemolysis on rabbit blood agar; gelatin was liquefied; growth was slightly yellow on agar slant. The organism was aerobic and growth was prevented by bile salts. Litmus milk was alkaline, reduced, peptonized and coagulated. Indol, acetyl-methyl-carbinol and hydrogen sulfide were not produced. Nitrates were not reduced. Methyl red and citrate agar tests were negative. No acid or gas was produced in peptone-carbohydrate broths, but in a synthetic carbohydrate medium many sugars were fermented. This bacillus was similar to the soil organisms studied by Conn.

Wastl (1945) reported four cases of enteric fever in which blood cultures showed Bacterium faecalis alkaligenes and the patients serum contained agglutinins for the isolated organism in titers varying from 1:50 to 1:1500. The author also found eight blood cultures positive for this organism in which agglutinins were less than 1:50 or had no titer at all. (The organism was not described.)

Anderson (1945) described a new member of the genus Alcaligenes which differed culturally and physiologically from other described species of the genus. The outstanding characteristic of this organism was its pronounced acid tolerance. It was a small gram negative rod, which produced a sparse growth on the common culture media as compared with Alcaligenes

faecalis. Growth on nutrient agar was poor. Broth growth was sparse with a granular precipitate, the supernatant liquid being clear. Gelatin was not liquefied; litmus milk was rendered alkaline, without coagulation, and was very slowly peptonized; no growth on potato. Carbohydrates and citrate were not utilized; nitrates were not reduced; indol and pigment was not produced and blood was not altered. (No mention is made of motility or flagellation.)

Altmeier (1945) reported the marked sensitivity of five strains of Bacillus alkaligenes faecalis to penicillin. (No description of the organisms was given.)

Fulton et al. (1946) reported that the findings of Altmeier could not be confirmed. Despite the fact that the genus Alcaligenes was separated from the Enterobacteriaceae and grouped with other genera in the family Rhizobiaceae, it occurred with, and resembled physiologically, the members of the former family. The resemblance includes insensitivity to penicillin.

Oeding (1946) isolated Alcaligenes hemolysans from the eyes of a new born. The child suffered from a moderate purulent conjunctivitis in both eyes. The strain was described and found to be in accordance with Henriksen's (1937) description. In addition to the case described above the author found 90 cases in which Alcaligenes was isolated from clinical material and entered in the laboratory diaries during the preceeding 7.5 years. Organisms routinely were classified as Alcaligenes if they were found to be gram negative rods, which did not ferment sugars or form pigment. The author pointed out the possibility of mistaking weak pigment producing Pseudomonas strains as Alcaligenes.

Dick (1946) found Bacterium faecalis alkaligenes in the blood in

five cases in which enteric group organisms were also isolated from blood, urine or feces. In three other cases, which were clinically enteric fever, Bacterium faecalis alkaligenes was isolated from the blood. (The organism was not described.)

Wilson and Miles (1946, page 786) observed that in the 5th edition of Bergey's Manual (1939), Bacterium bronchisepticus was included in the Brucella group, whose type species, Brucella melitensis it resembled in morphology and carbohydrate fermentation. This was in accordance with Evans (1918). Wilson and Miles point out the antigenic relationship between B. bronchisepticus and Haemophilus pertussis and suggest reclassification. Antigenic similarity alone does not necessarily indicate a taxonomic relationship, but in this case other resemblances are sufficiently good to warrant provisional inclusion of B. bronchisepticus in the same group as Haemophilus pertussis. The cultural relationships between Haemophilus pertussis and Brucella bronchisepticus are as close as those between Brucella bronchisepticus and Brucella melitensis.

Cossery (1946) isolated Bact. faecalis alcaligenes from blood of a patient with enteric fever. (The organism was not described.)

Idnani and Seetharaman (1946 and 1947) found Bacterium alcaligenes in dead duck embryos. It was a nonmotile, gram negative rod; on agar colonies developed lemon-yellow color; no liquefaction of gelatin; no change in glucose, lactose or sucrose broth; Voges-Proskauer, methyl red and indol reactions were negative; no reduction of nitrate; litmus milk was rendered alkaline; hydrogen sulfide was produced. They suggested this organism was Bacterium alcaligenes metalcaligenes (Castellani).



Spanedda and Aru (1946) studied 36 strains of motile and nonmotile Alcaligenes isolated from feces.

Rush (1947) in a dissertation presented to the faculty at Purdue University, on a taxonomic study of the mesophilic Achromobacter, observed that 5 serological groups of his collection of 405 organisms did not change litmus milk and did not produce acid in dextrose medium. All were said to have been stained by Gray's method for flagella and found to have peritrichous flagellation.

Terry et al. (1947) isolated Alcaligenes faecalis four times from spinal fluid and once from blood of a patient which seemed to establish bacteremia and acute meningitis due to this organism. The organism was a motile gram negative rod which did not produce acid or gas in carbohydrate media; litmus milk became alkaline; nitrates were reduced to nitrites and indol was negative. It was identified on this basis as Alcaligenes faecalis. Specific agglutinins for this organism were not found in the blood at any time.

Dyer (1947) studied microorganisms from Atlantic cod. She isolated five strains of Alkaligenes ammoniagenes and five strains of Alkaligenes metallkaligenes. They were assigned these names on the basis of the descriptions given in the 5th edition of Bergey's Manual. (The organisms were not described in the paper.)

Shrewsbury and Barson (1947) reported the isolation of 2 groups of organisms which appeared to be Alcaligenes. They were isolated from Arion ater (black slug). The motility varied but flagellar studies were not recorded.

Montestruc et al. (1947) reported they found Alcaligenes faecalis in dysentery stools. (The organisms were not described.)

Birchoff et al. (1948 and 1948) reported the isolation of Alkaligenes faecalis from 2 cases of bacteremia and meningitis in infants. The organism was described as a gram negative motile rod. Gelatin was not liquefied; indol, methyl red and Voges-Proskauer tests were negative. Dextrose and other listed carbohydrates gave a negative reaction; litmus milk was slowly alkalized.

Satum and Bourbon (1948) studied both Bacterium alcaligenes faecalis and Haemophilus bronchisepticus. These organisms had the same morphological and biochemical properties. They were motile with lophotrichous flagellation (with the exception of four strains); gram negative with no spores or capsules; gelatin was not liquefied; dextrose and other listed carbohydrates were not fermented; aerobic; hydrogen sulfide, indol, methyl red and Voges-Proskauer tests were negative; none were hemolytic for horse red blood cells; litmus milk was always turned blue. (The flagellation was not substantiated with photomicrographs.)

Shrewsbury and Barson (1948) reported the isolation of organisms similar to Bacterium alcaligenes from Periplaneta americana, the American cockroach.

Moustafa et al. (1948) isolated B. faecalis alcaligenes while studying food poisoning in Egypt. (The organism was not described.)

Quintos and Buensuceso (1948) reported the isolation of several strains of Alkaligenes during a study of diarrhea among Filipino infants. (The organisms were not described.)

Bergey's Manual of Determinative Bacteriology, 6th edition (1948)

describes Alcaligenes faecalis as follows:

Rods: 0.5 by 1.0 to 2.0 microns, motile with peritrichous flagella.

Gram negative.

Gelatin stab: No liquefaction.

Agar slant: White, glistening, opalescent, undulate margin.

Broth: Turbid, with thin pellicle, and viscid sediment. Gives off ammonia.

Litmus milk: Alkaline.

Potato: Scanty to abundant, yellowish to brownish growth.

Indol not formed.

Nitrite production from nitrates variable.

No acid or gas from carbohydrate media.

Aerobic, facultative.

Habitat: Intestinal canal. Generally considered nonpathogenic.

Alcaligenes faecalis var. radicans is listed as a gelatin liquefying strain.

Pulvirenti (1949) reported that B. faecalis alcaligenes produces hydrogen sulfide, however the other characteristics of the organism were not published.

Roland and Bourdon (1949), recorded that Alcaligenes fecalis gives a variable Ferguson-Hook urea reaction.

Hall and Garvan (1949) reported a fatal case of septicemia due to Bacterium faecalis alkaligenes. The motile gram negative bacillus was isolated from the blood in a febrile illness lasting for more than three months. Dextrose and other listed carbohydrates were not fermented, indol was not produced, litmus milk became alkaline. No agglutinins for the organism isolated were found in the blood of the patient. (The flagellar morphology was not recorded.)

Bassler and Peters (1949), without listing references, state Alcaligenes faecalis is found in about 20% of normal stools, with the percentage rising in intestinal inflammation. Vibrio alcaligenes appears normally in the intestinal tract and, as is common with other so-called intestinal

saprophytes, they are capable at times of producing an enteritis, and can be recovered from the blood in association with Salmonella.

Banerjee and Sarkar (1949) reported the isolation of Bacterium faecalis alkaligenes from blood in 27 cases; from ascitic fluid in 2 cases; from abscess pus in 2 cases; from knee joint fluid in 1 case; and from gall-bladder bile in 1 case. There were a few instances of nonagglutination of the isolated strain with the patients serum, however after repeated subculture the organisms agglutinated. (A description of the organisms isolated was not given.)

Olsen (1949) reported in his dissertation the isolation of Heliconema from 6 children. This peculiar organism could only be isolated under anaerobic conditions. The morphology presents a very heteromorphous picture, from rods which were straight to those which were spirochete-like. Vibrio forms as well as coccoid bodies were seen. The gram reaction was variable. The cultures soon after isolation became facultative anaerobes. Motility was never seen in any of the forms and flagella could not be demonstrated. Agar slant growth was thin, whitish, flat, glistening and undulate. Gelatin was not liquefied; bouillon became slightly turbid, some strains produced a little precipitate and ring. No growth on potato. Milk was unchanged; one strain gave a positive hydrogen sulfide test while the rest were negative; indol, urea, nitrate, methyl red and Voges-Proskauer tests were negative. Hemolysis of red blood cells did not occur. Glucose, sucrose and lactose gave no acid or gas formation. The systematic position of the organism was considered to be very indefinite.

Adamson (1949) reported he had isolated Alcaligenes faecalis at

autopsy from a clinical case of typhoid fever. The organism was present in the abdominal lymph nodes, spleen, trachea, bronchi, hilar, axillary and inguinal lymph nodes. Salmonella typhi was not found in any part examined. (The organism was not described.)

Severi (1949) isolated an organism resembling Vibrio alcaligenes from a thigh phlegmon. It was a gram negative rod, very actively motile, slightly curved with pointed ends, and polar flagella. Broth growth was homogeneous with a pellicle and sediment. The organism was aerobic; gelatin not liquefied; no hemolysis on blood agar was noted. Carbohydrate broths became alkaline, except glycerine, glucose, laevulose and dulcitol which remained unchanged. Nitrates were reduced to nitrites; litmus milk became alkaline and reduced. Hydrogen sulfide was produced. The author thinks a classification of the above bacterium is not yet possible and therefore it is convenient to maintain the term Vibrio alcaligenes to indicate Alcaligenes-like bacteria which are characterized by Vibrio morphology and are not identical with Agrobacterium.

Bitter and Williams (1949) reported the isolation of several strains of Alcaligenes from the hind gut contents of Periplaneta americana, the American cockroach. Organisms which did not ferment dextrose, lactose or sucrose were placed in the genus Alcaligenes. (These organisms were not described more fully.)

Carroll et al. (1949) published a report showing that Alcaligenes strains responded variously to streptomycin and aureomycin, with some strains sensitive, others resistant. Their results would indicate that aureomycin is effective in Alcaligenes urinary tract infections.

Simsonds and Fruton (1949) described an organism (SF) which utilized peptides and was identified as Alcaligenes. The organism was a short, nonmotile, gram negative rod. In nutrient broth containing glucose, lactose or sucrose it did not cause the formation of acid or gas; after 3 days, such test solutions had a pH of 8 or higher. Litmus milk became alkaline and gelatin was liquefied. Simsonds and Fruton (1951) with some suggestions by Einar Leifson described the organism more carefully. Strain SF was motile, polar flagellated, and predominantly monotrichous. The motility was best observed in cultures incubated at about 20°C.; cultures grown at 37°C. were practically nonmotile. In spite of the alkaline reaction in peptone carbohydrate media, a synthetic medium composed of inorganic salts, including phosphate and ammonium ions, the bacteria utilized glucose as a carbon source. Strain SF was more logically placed in the genus Pseudomonas, rather than Alcaligenes as suggested earlier.

Weinberg (1950) studied vitamin requirements of dwarf colony variants of Alcaligenes faecalis. (The physiological and morphological characteristics of the organism was not published.)

Miles (1950) found Bacterium alkaligenes responsible for red leg in frogs. The organism was a gram negative, nonacid-fast rod, feebly motile with peritrichous flagella. Horse blood agar colonies were yellowish and nonhemolytic; it failed to ferment a wide range of carbohydrates. Citrate, indol, methyl red, Voges-Proskauer, catalase, ammonia and hydrogen sulfide tests were negative. Gelatin was not liquefied; nitrates were not reduced to nitrites; litmus milk became alkaline. The organism was not agglutinated by antiserum to authentic strains of Haemophilus bronchisepticus.

Wrinkle et al. (1950) found Alcaligenes to be one of the principal genera of bacteria in pasteurized and unpasteurized eggs and egg products.

Elyan and Mustafa (1950) in Egypt isolated, from materials obtained during food poisoning, B. faecalis alkaligenes on Mifco S. S. agar.

Wirtz (1950), while in Cairo, Egypt during World War II reported the isolation of B. faecalis alkaligenes from flies captured in mess halls.

Coffey et al. (1950) and Garvey et al. (1950) reported studies with chloramphenicol concerning in vitro resistance and treatment of urinary tract infections by Alcaligenes.

Kirby et al. (1950) reported the isolation of 5 strains of Bacterium alkaligenes in a study of neonatal diarrhea and vomiting.

Castellanos et al. (1950) reported the isolation of Alcaligenes along with Escherichia and Proteus from mesenteric lymph nodes of a child who died of acute diarrhea.

Gomes et al. (1950) isolated Alcaligenes fecalis from infants.

Mushin (1950) studied gastro-enteritis in a children's hospital in Melbourne. Among other organisms Bacterium alkaligenes faecalis was isolated twice from abnormal stools. One strain was isolated in association with Proteus mirabilis and the other one was the only nonlactose-fermenter present in the fecal specimen. Both strains were actively motile on isolation.

Signorine (1951) made 65 stool cultures of breast fed infants with enteric disturbances. A Shigella was isolated once, a Salmonella three times and Fecalis alkaligenes 12 times.

Weinstein and Wasserman (1951) presented a case with infectious mononucleosis and Bacterium alkaligenes bacteremia complicated by fuso-

spirochetal angina. The isolated organism was described as a gram negative rod with sluggish motility. It produced no hemolysis, no indol; litmus milk became alkaline, and a dark brown pigment was produced on potato. Dextrose and other listed carbohydrates were not fermented. Using as antigen the isolated strain, the serum titer of the patient rose as follows: 1:32 (admission), 1:128 (10 days later), 1:512 (4 weeks after onset of bacteremia).

Leifson (1951) published photomicrographs of several strains of Alcaligenes showing the peritrichous and lophotrichous flagellation very clearly.

Wynne et al. (1952) reported the isolation of Alcaligenes from cloudy cerebrospinal fluid aspirated from a three year old child at necropsy. The organism was a motile gram negative rod which failed to ferment glucose, lactose or sucrose. Hydrogen sulfide production and gelatin liquefaction tests were negative. This organism was also isolated from the blood and colon. Shigella paradysenteriae II was also isolated from the colon.

Jensen (1951) studied 3 Alcaligenes strains, isolated from Brazilian soil, in respect to nitrification of oxime compounds.

Eldering and Kendrick (1952) differentiated Haemophilus pertussis, the parapertussis bacillus, and Brucella bronchiseptica by the characteristics given in the table below. Since these organisms are misfits in the genus Haemophilus and antigenically related it was suggested they be grouped together in some other genus.



	<u>H. pertussis</u>	<u>parapertussis</u> <u>bacilli</u>	<u>Br. bronchiseptica</u>
growth on blood-free peptone agar	-	+	+
"browning" of peptone medium	-	+	-
motility	-	-	+
ability to split urea	-	++	+
citrate utilization	-	+	+

\* occasionally negative

Shrewsbury and Barson (1952) isolated from a house sparrow an organism which was devoid of any saccharolytic activity under the test conditions.

Sir Aldo Castellani (personal communication 1952) proposed the following taxonomic scheme for the new edition of the Manual of Tropical Medicine (1919) not yet published.

GENUS ALCALIGENES Castellani and Chalmers, 1919.

Definition: Eberthese which do not ferment glucose or lactose, and are characterized by their general lack of fermentative power, producing neither acidity nor gas, and by actually increasing the alkalinity of the media.

Milk is not clotted, and is rendered alkaline.

Type: Alcaligenes faecalis (Petruschky, 1896), emendavit Castellani and Chalmers, 1918.

Classification: These various organisms can be differentiated as follows:

- A. Grows well on ordinary media. Rod-like organisms, not coccoid, little or no pathogenicity:  
 Subgenus Alcaligenes.  
 (a) Motile:  
 I. No odour, faecalis.  
 II. Foetid or putrid odour, alkalofaecidus.  
 (b) Nonmotile: metalkalofaecidus.
- B. Grows scantily and very slowly on ordinary media. Very short rods, almost coccoid. Causes undulant fever:  
 Subgenus Brucella  
 I. Habitat goats and sheep, melitensis.  
 II. Habitat cattle, abortus.  
 III. Habitat swine, suis.

Castellani's Spirillum seylanicum (1910) was placed in the genus Vibriothrix. Castellani states that organisms belonging to this genus are often mistaken for Alcaligenes.

Ulrich and Needham (1953) reported the differentiation of Brucella bronchiseptica and Alcaligenes faecalis by biochemical and nutritional methods. (The flagellar morphology of the organisms studied was not recorded by the authors. It can be seen from the data presented that Ulrich and Needham strains 212, 249, 282, 4741 and 8749 correspond respectively to RH strains 137, 430, 431, 377 and 175. These strains can not be considered as typical Alcaligenes faecalis for one or more of the following reasons, 1) production of acidity from carbohydrates, 2) polar multitrichous flagellation, 3) normotility.)

Alcaligenes viscosus

Adametz (1889) isolated, from Vienna brook water, a capsulated organism which turned milk slimy. The capsule increased in size when the organism was grown in milk. It was a slightly motile rod occurring singly and occasionally in twos. The colony on agar media appeared slimy. Milk cultures also became slimy, ropy and slightly alkaline without odor or precipitation of casein. Slime was produced in peptone salt water. The organism was aerobic and was named Bacillus lactis viscosus. (There is no record of size, gram reaction, flagellation or action on carbohydrates.)

Adametz (1891) observed this same organism in milk from Switzerland. The organism was traced to well water. It was motile, without spores, did not liquefy gelatin stab but caused milk to become ropy. Sucrose and lactose were not attacked and no gas was produced in milk.

Marshall (1896) in a paper on ropiness in cream and milk described an organism under the heading "The Bacillus Causing the Trouble." (No name was given.) The organism was a short, thick, nonmotile bacillus 2 by 1.25 microns. No spores were detected (gram reaction was not recorded). Gelatin was not liquefied. The culture on agar slant was whitish, slimy and ropy. Potato growth appeared yellowish white. Bouillon strung out when the needle was applied. Acidity of milk decreased slightly but no odor was discernible. Ability to produce ropiness was lost when cultured on laboratory medium.

Flügge (1896) listed this organism as Bacillus viscosus lactis (Adametz).

Chester (1897), in a classification of the Schizomycetes, characterized Bacterium viscosus lactis (Adametz) as nonmotile, capsulated, asporogenous and gram positive. The organism was not strictly aerobic and did not liquefy gelatin. Milk became viscous, peptonization and clearing occurred with no odor. Agar colonies were dirty white and slimy. Habitat milk. (Carbohydrate reaction was not recorded.)

Lehmann and Neumann (1899) characterized Bacterium lactis viscosum (Adametz) as nonmotile, gram positive, capsulated. Grape-sugar and milk-sugar were not fermented; little indol and no hydrogen sulfide was produced. The authors state that their culture, obtained from Kral, nothing was to be seen of the spore formation which Zimmermann claimed to have seen. The organism was discovered by Adametz as an important enemy of the butter industry. Zimmermann found this organism in water.

Conn (1899) in his classification of dairy bacteria described B. viscosus lactis II (n. sp.). It was a rod four times as long as broad (size not reported), surrounded by a mass of slime. It did not liquefy gelatin. Agar growth was slimy; potato growth was grayish brown. Milk became slimy and alkaline with an odor reminding one of strong cheese. This bacillus, the author stated was physiologically similar to B. viscosus lactis of Adametz, but its morphology was quite different. While B. viscosus was nearly as broad as long, this organism was long and slender. (There was no mention of gram stain, motility, flagellation or carbohydrate reaction.)

Ward (1899) reported on ropiness in milk and cream. He was of the

opinion that the organism described by Marshall, which was responsible for the ropy milk outbreak in Michigan, could not be positively identified from the brief description available, but there was reason to suspect its identity with Bacillus lactis viscosus. He described an organism which was isolated from water and milk and probably identical with Bacillus lactis viscosus Adametz (1889). This rather complete description stated the organism to be gram positive.

Migula (1900) used the name Bacterium subviscosum for the organism Bacillus lactis viscosus Adametz, but used the description recorded by Zimmermann. The gram positive, nonmotile organism was described as forming spores which had not been observed to germinate; gelatin was not liquefied. Migula also described Bacterium viscosum (Kramer) Mig. It was a rod 1 by 2.5 to 4 microns, nonmotile. Gelatin was liquefied and slimy growth occurred on carrot slices.

Conn et al. (1906) pointed out that the organisms producing slimy milk "do not form a class by themselves, for this property of rendering milk slimy seems to be found widely scattered among the bacteria".

Buchanan and Hammer (1915) in their study of slimy and ropy milk list Bacterium lactis viscosum (Adametz) Lehmann and Neumann as synonymous with:

Bacillus lactis viscosus, Adametz  
Bacillus viscosus lactis, Conn  
Bacillus viscosus lactis II, Conn  
 Group I of Harrison, Numbers 1, 2, 3 and 4.  
Bacterium lactis acidii, Marple

These authors consider the organism to be a gram negative, nonmotile, asporogenous rod. The organism did not produce acid or gas from any

carbohydrate." They propose the name Bacterium visco-coccoidium sp. nov., and give it as synonymous with Group IV, varieties 9 and 10 of Harrison's slimy milk bacteria, for an organism which produced acid from dextrose, lactose and maltose and formed slimes in milk. (Bacterium visco-coccoidium is still retained in Bergey's Manual of Determinative Bacteriology, 6th edition (1948) as a synonym for Alcaligenes viscosus.)

According to Magnusson (1918), a culture of a sugar fermenting organism named Bacillus lactis viscosus Adametz, and assumed to be Bacterium lactis viscosum Lehmann and Neumann, was for a considerable period distributed from the Kral collection.

Weldin and Levine (1923) named the organism Bacterium viscosum and placed it in the subgenus Alcaligenes. It was a capsulated, asporogenous, gram negative rod, nonmotile or motile by peritrichous flagella. No acid or gas was produced from glucose. Milk was rendered slimy and gelatin was not liquefied.

Bergey's Manual of Determinative Bacteriology (1923) used the name Lactobacillus viscosus (Adametz) with probable synonyms: Bacillus viscosus lactis, Conn; Bacillus viscosum lactis II; Bacterium lactis acidii, Harpmann; Bacterium visco-coccoidium, Buchanan and Hammer. It is described as having no action on carbohydrates, nonmotile and gram negative. Bergey also used the name Alcaligenes metalcaligenes (Adametz) and cites Adametz's work of 1891. It was described as a gram positive, indol producing organism.

Bergey's Manual of Determinative Bacteriology, 2nd edition (1925) carries the name of this organism as Achromobacter viscosum (Adametz) with the same description which appeared in the previous edition.

Weldin (1927) includes Alcaligenes viscosum (Adametz) comb. nov. in his taxonomy. It was synonymous with:

Bacterium viscosum (Adametz) Weldin and Levine, 1923  
Bacillus lactis viscosus Adametz, 1889  
Bacillus viscosus lactis (Adametz) Krause, in Flugge, 1896  
Bacterium viscosus lactis (Adametz) Chester, 1897  
Bacterium lactis viscosum (Adametz) Lehmann and Neumann 1901  
Lactobacillus viscosus (Adametz) Bergey et al. 1923  
Achromobacter viscosus (Adametz) Bergey et al. 1925

Weldin states that Adametz considered this organism to be gram negative, but more resistant to decolorization than related species.

Lehmann and Neumann (1927) continued to list the organism as Bacterium lactis viscosum (Adametz).

Long and Hammer (1935) reported the isolation of fat-splitting bacteria in normal and abnormal (but not ropy) milk, cream and other similar products. Thirty six of these cultures showed the characteristics of Alcaligenes viscosus, including ropiness in milk. Carbohydrates in bouillon were attacked only slightly if at all. A few of the cultures produced acid, but no gas, from certain of the sugars but later reversed the reaction. They were aerobic, nonmotile, asporogenous rods, 0.6 to 1 by 0.8 to 2.6 microns. They were essentially gram negative but in certain cultures there were a few cells showing a tendency to retain the gram stain. Capsules were produced in milk cultures. Agar growth was white. Gelatin was not liquefied. Litmus milk became ropy, alkaline, without coagulation or proteolysis. The following reactions were negative: indol, nitrate reduction, hydrogen sulfide production, methyl red and Voges-Proskauer. There were 25 cultures which were like Alcaligenes viscosus, except for the failure to produce ropiness and were considered to be nonropy strains of Alcaligenes viscosus. They were designated

Alcaligenes viscosus var. dissimilis.

Druce and Aynsley (1949) made some observations on ropy milk in Lancastershire, England. Cultures isolated were found to correspond in many respects to Alcaligenes viscosus (Bergey 1948). This organism differs from Bergey's description in the type of growth on broth and agar.

Abd-el-malek (1952) found that Alcaligenes viscosus showed lipolytic activity. This confirmed the report by Long and Hammer (1935). Some of the strains studied by Abd-el-malek reduced nitrate. Citrate oxidation and slime production did not appear to be identifying characteristics of Alcaligenes viscosus.



Alcaligenes metalcaligenes

Castellani and Chalmers (1919) listed an organism in their taxonomic scheme which was differentiated from Alcaligenes faecalis (Petruschky 1896) by its nonmotility. The organism was listed as Alcaligenes metalcaligenes from the organism named B. meta alkaligenes described by Castellani. This organism was described as being nonmotile, gram negative, gelatin and coagulated serum not liquefied. Alkaline reactions were observed in glucose, maltose, lactose and sucrose. Indol was variable and broth showed a general turbidity.

Bergey's Manual of Determinative Bacteriology (1923) lists this organism as follows: Alcaligenes metalcaligenes (Adametz). Synonymous with Bacillus metalcaligenes and Bacterium lactis viscosum Adametz (1891). Rods 0.5 by 1.0 to 1.5 microns, occurring singly and in pairs, nonmotile, gram positive, encapsulated. Growth on agar slant moist, smooth, spreading, slimy; broth becomes turbid, with slimy sediment; litmus milk becomes thick, slimy, but not coagulated. Potato growth is gray, moist, spreading, slimy; indol is formed; acid in dextrose. Not pathogenic. Aerobic. Found in slimy milk.

Weldin and Levine (1923) in their key to the species and varieties of intestinal bacteria list this organism as Bacterium metalcaligenes in the subgenus Alcaligenes. This organism was stated to be nonmotile and not liquefying gelatin, not forming capsules in milk nor turning it slimy.

Bergey's Manual of Determinative Bacteriology, 2nd edition (1925)

listed the organism as Achromobacter metalcaligenes.

Levine and Soppeland (1926) isolated Bacterium metalcaligenes Castellani and Chalmers, from creamery wastes, and which had the following characteristics; gram negative, asporogenous, nonmotile, aerobic rods. Heavy pellicle in broth, no liquefaction of gelatin or Loeffler's serum, milk becomes alkaline without coagulation or peptonization, litmus reduced in 10 days, nitrate reduction variable, no gas. Hydrogen sulfide and indol was not produced. No acid or gas from glucose, lactose, sucrose or glycerol peptone water.

Weldin (1927), in his taxonomic scheme listed this organism as Alcaligenes metalcaligenes Castellani and Chalmers (1919).

Bergey's Manual of Determinative Bacteriology, 3rd edition (1930) removed the Adametz reference from the description of the organism for it appeared as Alcaligenes metalcaligenes Castellani and Chalmers, (1919). (There is no reference as to the source of the description.)

Bergey's Manual of Determinative Bacteriology, 6th edition (1948) describes Alcaligenes metalcaligenes Castellani and Chalmers as follows:

Rods: 0.6 by 1.5 microns, with rounded ends, occurring singly and in pairs.  
Normotile. Gram negative.  
Gelatin stab: No liquefaction.  
Agar colonies: Circular, raised, smooth, amorphous, entire, gray.  
Broth: Membranous pellicle with heavy sediment.  
Litmus milk: Alkaline.  
Potato: Scanty, glistening, smooth, sometimes faint pink.  
Indol not formed.  
Nitrite production from nitrates variable.  
Starch not hydrolyzed.  
Blood serum not liquefied.  
No action on carbohydrates.  
Aerobic, facultative.  
Optimum temperature 22°C.  
Habitat: Intestinal canal.

Allen and Brooks (1949) reported they recovered Bacterium metalcaligenes (Levine and Soppeland, 1926) from chlorine treated sewage

Elliker et al. (1952) reported Alcaligenes metalcaligenes produces a marked gelatinous film around the curds of cottage cheese at low temperature. (The organism was not described.)

### Alcaligenes bookeri

Booker (1887) was the first to describe an organism which has since been included in the genus Alcaligenes. He reported the isolation of Bacillus A from alvine discharge of children suffering with cholera infantum. The organism was actively motile in hanging drop; milk became coagulated, alkaline and peptonized; it liquefied gelatin and grew on potato with a brown color. (There was no mention of carbohydrate reactions, gram stain, or flagellar morphology.) Booker (1890) recognized that Bacillus A may have been Proteus vulgaris. Sternberg's (1893) Manual of Bacteriology included Booker's description of Bacillus A.

Ford (1903) isolated and studied an organism, from the stomach of a foundling, which he considered to be the same as Bacillus A and named it Bacillus bookeri, Ford. He added the following characteristics to Booker's description. Rods with no spores, no acid or gas produced from dextrose, lactose or sucrose. In a Smith tube containing dextrose broth growth was limited to the open bulb. (No gram reaction or flagellar morphology was included.) Indol was not produced. Abundant yellow or yellow-brown growth appeared on agar slant. Litmus milk became alkaline and reduced but no coagulation occurred. (It should be noted that the milk reaction and pigmentation in this report were variously given.) Nitrates were not reduced to nitrites. Gelatin, casein and coagulated blood serum were liquefied.

Levine and Soppeland (1926) when studying bacteria of creamery wastes added characteristics to the organism they called Bacterium bookeri (Ford). This organism was gram negative, potato growth was abundant in 48 hours but with pale olive color; slight reduction of nitrates to nitrites with no gas production observed. The name Bacterium bookeri var. immobilis, nov. var., was coined for the nonmotile variant.

Bergey's Manual of Determinative Bacteriology (1923) listed this organism as Alcaligenes bookeri (Booker), motile by means of peritrichous flagella, gram negative and nitrates not reduced.

Weldin's (1927) taxonomic scheme lists this organism as Alcaligenes bookeri (Ford) Bergey et al. (1923).

Kutscher (1937) isolated from spinal fluid an organism which he called Alcaligenes bookeri. It was a small, motile gram negative rod. Growth on plain agar was abundant; growth on potato was abundant and brown; gelatin was liquefied; litmus milk was reduced and alkaline in 24 hours with clearing in the upper portion. No fermentation of any carbohydrates including glucose, mannitol and xylose, which became alkaline. Indol, methyl red and Voges-Proskauer tests were negative and nitrates were not reduced.

Bergey's Manual of Determinative Bacteriology, 6th edition (1948) continues to describe Alcaligenes bookeri as being motile with peritrichous flagella. Nitrates were not reduced to nitrites.

Alcaligenes recti

Ford (1903) in a report on the classification and distribution of intestinal bacteria in man, described Bacillus recti, (n. sp.). This organism was an actively motile, asporogenous rod 0.5 by 1.5 to 2.0 microns, gelatin was liquefied. Dextrose, lactose and sucrose were not fermented. In a Smith tube containing dextrose broth growth was limited to the open bulb. Nitrates were reduced to nitrites, indol was not produced, litmus milk became alkaline.

Bergey's Manual of Determinative Bacteriology, 6th edition (1948) continues to list this organism as Alcaligenes recti (Ford) Bergey et al. The description given by Ford was used with the addition of two fundamental characteristics, peritrichous flagellation and gram reaction, which were not recorded by Ford or subsequent workers.

Alcaligenes marshalli

Marshall (1904) reported the isolation of a peptonizing bacterium from milk, but did not describe the organism.

Conn et al. (1906) published a classification of dairy bacteria and stated they had studied the organism which Marshall isolated. It was a gram negative asporogenous rod without capsule. (There is no mention of flagellation or motility.) Gelatin was liquefied; no acidity, gas or closed arm growth in Smith fermentation tubes (carbohydrates tested were not listed). Agar streak growth was viscid, smooth, cream-white becoming lemon-yellow. Milk became slimy, alkaline and digested without coagulation. Potato growth was lemon-yellow. (There was no mention of indol or nitrate reactions.) The authors were of the opinion that essentially the same organism was found in Connecticut and New York except it differed in that milk was not digested.

Buchanan and Hammer (1915) in a report on slimy and ropy milk listed Bacterium marshalli Conn, with all the characteristics reported by Conn et al. (1906), but recorded there was no motility or acid produced from any sugars.

Bergey's Manual of Determinative Bacteriology, 6th edition (1948) recorded that Alcaligenes marshalli Bergey et al. was nonmotile, no acid from any sugars, indol not formed, and nitrates not reduced.

### CHAPTER III

#### COMMENTS ON THE HISTORY

If the genus Alcaligenes is to be characterized as having peritrichous flagella several species (bookeri and recti) now recognized in the genus Alcaligenes in Bergey's Manual of Determinative Bacteriology, 6th edition (1948) should be deleted. Neither the original description, nor any subsequent descriptions, of these organisms have included the nature of the flagellation. Actually the lively motility recorded for bookeri and recti are more suggestive of polar than peritrichous flagellation.

Cultures of Alcaligenes recti and Alcaligenes marshalli are not found in the American Type Culture Collection, the National Collection of Type Cultures, nor could they be obtained from private collections. Two cultures labeled Alcaligenes bookeri were studied (RH 21 and 229) and both were found to be polar flagellated.

Convincing evidence has not been offered which indicates that Alcaligenes marshalli should be nonmotile. Buchanan and Hammer (1915) state that Bacterium marshalli is nonmotile, however the description given was that of Conn et al. (1906), to which was added a statement regarding nonmotility. It is suggested that Alcaligenes marshalli no longer be recognized, since it has not been adequately described. It is suggested that the names Alcaligenes bookeri, recti and marshalli become known as nomina dubia since



their application is uncertain.

Castellani and Chalmers (1919) described Alcaligenes metalkaligenes and stated the name was taken from B. meta alkaligenes which appeared in a paper by Castellani (1915). The original reference has not been found. Doctor Robert Breed (personal correspondence 1952) was likewise unable to locate the original publication. Sir Aldo Castellani (personal correspondence 1952) was unable to give the exact documentation since his reprints were lost during the war, however he thought the organism was first mentioned and its characteristics given in a lecture he delivered at Taranto, Italy, in 1915. He thought the lecture and/or a report of the organism was published in several Italian medical journals.

The description by Adametz of Bacillus lactis viscosus does not include the gram reaction. Several taxonomists such as Chester (1897), Lehmann and Neumann (1899), and Migula (1900) considered this organism to be gram positive. Ward's (1899) experience indicated the organism to be gram positive and recognized the earlier description of Bacillus lactis viscosus Adametz was incomplete for precise reidentification. Buchanan and Hammer (1915) were of the opinion that many of the organisms described earlier, such as Bacillus lactis viscosus, Adametz, Bacillus viscosus lactis, Conn, and Bacillus viscosus lactis II, Conn, though lacking a statement of the gram reaction in the original description, were gram negative. Generalizations as these can only be accepted, at best, with considerable reservation.

More recently Long and Hammer (1935) studied many organisms capable of producing ropiness in milk. Although these organisms were gram negative and considered to be Alcaligenes viscosus, the comments concerning their

effect on dextrose bouillon indicates they were capable of oxidizing carbohydrates. All this can only mean that his strains included in Alcaligenes as viscosus were far from being homogeneous.

Adametz (1891) who is generally credited with the original characterization of Alcaligenes viscosus found it to be slightly motile. Workers since have consistently reported this organism as being nonmotile. Abd-el-malek suggests that this indicates that Adametz was in error. Bergey's Manual of Determinative Bacteriology (1948) continues to refer to this report concerning motility.

As noted in the review of the literature, several spellings are used to denote the genus Alkaligenes. The spelling Alcaligenes is probably correct. The Germans in transliterating from the Greek frequently use a "k", as in kokkus, but in taxonomic work all names are latinized and should be transliterated as "c". Fortunately Petruschky in his first proposal of the name of the species regarded as the type, used the spelling Bacillus faecalis alcaligenes. Castellani and Chalmers (1919) used both spellings indiscriminately.

The problem of whether or not bronchicanis Ferry (1911) should be substituted for bronchiseptica Ferry (1912) is not simple. It raises the question of whether the author of an unfortunate name has the right to correct his error. Under the older Botanical Rules, usage would be followed rather than priority. Doctor Breed, editor of Bergey's Manual of Determinative Bacteriology, has preferred to use the almost universally accepted name to avoid changes in nomenclature. This appears to be good judgement while waiting for the formulation of a policy acceptable to the International Judicial

Commission on Bacteriological Nomenclature. Be this as it may, Chapter III, Section 1, Article 16 of the International Rules of Botanical Nomenclature, as accepted by the First International Microbiological Congress in Paris 1930, reads as follows: An organism "can bear only one valid name, the earliest that is in accordance with the Rules of Nomenclature". Chapter III, Section 12, Article 59 reads as follows: "A name or epithet must not be rejected, changed or modified merely because it is badly chosen, or disagreeable, or because another is preferable or better known."

Based on the above historical study the author would recommend that:

1. The designations Alcaligenes metalcaligenes, bookeri, recti and marshalli be rejected since their application is uncertain (nomina dubia).
2. The designation Alcaligenes viscosus be limited to gram negative rods which do not oxidize or ferment carbohydrates.
3. The species designation bronchiseptica be rejected for the epithet bronchicanis, Ferry (1911), which has priority.
4. The spelling Alcaligenes become universally accepted as the genus designation.

CHAPTER IV  
MATERIALS AND METHODS  
Bacterial Cultures

Two sets of stock cultures were maintained throughout the study. One set was kept at 8°C while the second set, with rubber stoppers, was kept at room temperature. The following semisolid medium was used:

beef extract	.3%
tryptone	.5
pH 6.9	

The medium was dispensed in 3-4 ml. amounts in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. The stock cultures were transferred every 3-4 months, incubated at 30°C until good growth appeared and then replaced in storage.

The temperature of incubation of the inoculated media was at 30°C. Generally there was better growth at 30°C than at 37°C for the majority of the organisms studied.

Morphology

GRAM STAIN

I. Ammonium Oxalate Crystal Violet (Hucker's)

Solution A		Solution B	
crystal violet (90% dye content)	2 g.	ammonium oxalate	0.8 g.
ethyl alcohol (95%)	20 ml.	distilled water	80 ml

Mix solutions A and B.

## II. Iodine Solution (Kopeloff and Beerman's)

iodine	2 g.
normal NaOH (40 g. per liter)	10 ml.

After iodine is dissolved, make up to 100 ml. with distilled water.

## III. Decolorizer

Equal volumes of 95% ethyl alcohol and acetone.

## IV. Counterstain (Burke's)

safranin O (85% dye content)	2 g.
distilled water	100 ml.

1. Dry thinly spread films in air without heat.
2. Flood with crystal violet. Allow to stand 1 minute.
3. Wash in tap water. Shake to remove excess water.
4. Cover with iodine for 2 minutes.
5. Wash in tap water. Shake to remove excess water. Do not blot or allow to dry.
6. Add decolorizer to the slide drop by drop until the decolorizer flows colorless from the slide.
7. Wash in tap water. Shake to remove excess water.
8. Counterstain.
9. Wash in tap water and dry.

Smears for gram staining were made from 18-24 hour agar slant growth.

## FLAGELLA STAIN

The technique for studying flagella was outlined by Leifson (1951).

It is believed the taxonomic criterion of significance should be the type of flagellation or absence of flagella rather than motility or nonmotility since nonmotile flagellated organisms have been reported.

## Physiology

CARBOHYDRATE UTILIZATION

pancreatic digest of casein (Bacto-Casitone)	0.5%
agar	0.2
carbohydrate	1.0
bromthymol blue (1.6% aqueous solution)	0.2 ml.
final pH 7.1	

Ten percent carbohydrate was Seitz filtered and added aseptically to the melted base which had been autoclaved at 121°C for 15 minutes. The medium was then dispensed aseptically in 4 ml. amounts into sterile 13X100 mm. tubes. All media prepared in this manner were tested for sterility by allowing them to stand overnight at room temperature. Each organism was inoculated by stabbing into duplicate tubes of the base medium without carbohydrate and duplicate tubes of the carbohydrate medium. One of each pair of duplicate tubes was layered with sterile melted petrolatum to a depth of 2 cm. This was found to be the most efficient practical method of excluding air. The tubes were checked for changes of indicator every 24 hours for 7 days.

INDOL

pancreatic digest of casein (Bacto-Casitone)	1.0%
pH 7.1	

The broth was dispensed in 13X100 mm. tubes, 4 ml. per tube, and autoclaved at 121°C for 15 minutes. The medium was inoculated in duplicate and tested for indol production after 24 and 48 hours. Modified Kovacs' reagent was used to test for indol production.

paradimethylaminobenzaldehyde	5 g.
isoamyl alcohol (Baker and Adamson 1210)	75 ml.
hydrochloric acid, concentrated	25 ml.

Aerobacter aerogenes and Escherichia coli were used as control organisms.

#### METHYL RED AND VOGES-PROSKAUER TESTS

Bacto MR-VP medium was used.

Methyl red test: Four ml. of broth was dispensed in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. After inoculation and 5 days incubation the reaction of the medium was tested with 5 drops of methyl red solution (0.1 g. methyl red in 300 ml. 95% ethyl alcohol and diluted to 500 ml. with distilled water).

Voges-Proskauer test: One ml. of the broth was dispensed in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. After inoculation and 2 days incubation, 0.6 ml. of 5% alpha naphthol in absolute ethyl alcohol and 0.2 ml. of saturated sodium hydroxide were added to determine the presence of acetylmethylcarbinol. Aerobacter aerogenes and Escherichia coli were used as control organisms for these tests.

#### CITRATE

Bacto Simmons Citrate Agar was used.

Three ml. of the medium was dispensed in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. The tubes were slanted, lightly inoculated and indicator change recorded after 24 and 48 hours. Escherichia coli and Aerobacter aerogenes were used as control organisms.

#### UREA

Bacto Urea Broth Concentrate was used.

This medium is prepared according to the formula of Rustigian and

Stuart (1941) and Stuart, van Stratum and Rustigian (1945). The concentrate was brought to volume with distilled water and aseptically dispensed in 2 ml. amounts in sterile 13X100 mm. tubes. Heavy inoculation was made from an 18-24 hour agar slant culture. Results were recorded after 24 and 48 hours incubation. Proteus morgani and Escherichia coli were used as control organisms.

GELATIN (Four media were compared.)

Medium 1	
gelatin	12.0%
pH 6.8	

The medium was dispensed in 5 ml. quantities in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. The medium was stabbed, incubated and tested for liquefaction each day, beginning with the second day, for 15 days by inverting the tube after cooling at 8°C for one hour.

Medium 2	
beef extract	0.3%
pancreatic digest of casein (Bacto-Casitone)	0.5
gelatin	12.0
pH 7.0	

This medium was handled as gelatin medium 1 above.

Medium 3	
yeast extract	0.3
tryptone	1.0
gelatin	12.0
pH 6.9	

This medium was handled as gelatin medium 1 above. The results recorded in Table II are based on this medium.

Medium 4	
Bacto Stone Extract Gelatin Agar	
gelatin	3.0%
beef extract	3.0



agar  
pH 6.8

1.5

The medium, described by Stone (1933) was dispensed in 2 ml. quantities in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. The tubes were slanted without butts. The medium was inoculated by streaking and incubated for 48 hours. Saturated (room temperature) ammonium sulfate was then added to cover the slant. Results were recorded as positive if the medium remained clear after exposure to the ammonium sulfate. Proteus vulgaris and Salmonella typhosa were used as control organisms in these gelatin tests.

#### HYDROGEN SULFIDE

Bacto Kligler Iron Agar was used.

The medium was dispensed in 4 ml. quantities in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. Tubes were slanted with a deep butt. The medium was inoculated from a broth culture by stabbing and streaking. Proteus morgani, Salmonella typhosa and Escherichia coli were used as control organisms.

#### NITRATE REDUCTION TO NITRITE

Medium 1

yeast extract	0.3%
pancreatic digest of casein (Bacto-Casitone)	0.5
agar	1.5
pH 7.0	

The medium was dispensed in 8 ml. quantities in 15X125 mm. tubes, autoclaved at 121°C for 15 minutes and then slanted.

Medium 2

pancreatic digest of casein (Bacto-Casitone) 0.5%

potassium nitrate  
pH 7.0

0.1

Medium 2 was dispensed in one ml. quantities in 10X75 mm. unsterilized tubes. The 18-24 hour growth from Medium 1 was heavily inoculated into Medium 2 and allowed to incubate in a 37°C water bath for 1 hour. The presence of nitrite was detected by adding 3 drops each of 8% dimethylalpha-naphthylamine in 5N acetic acid, and 5% sulfanilic acid in 5N acetic acid. The negative tubes were tested with zinc dust for the presence of unreduced nitrate.

#### NITRATE REDUCTION TO GAS

pancreatic digest of casein (Bacto-Casitone) 1.0%  
yeast extract 0.3  
potassium nitrate 0.2  
pH 7.0

The medium was dispensed in 8 ml. quantities in 15X125 mm. tubes with inverted vials to collect evolved gas. The medium was autoclaved at 121°C for 15 minutes. Gas was recorded after 1, 2 and 3 days incubation.

#### NITRATE AS A SUBSTITUTE FOR OXYGEN FOR GROWTH

Medium number	1	2	3
pancreatic digest of casein (Bacto-Casitone)	1%	1%	1%
potassium nitrate	0	0	0.1
pH 7.1			
petrolatum cover	+	-	+

The medium was dispensed in 5 ml. quantities in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. Macroscopic relative growth in the above media was compared at the end of 18 hours growth.

MANGANESE PSEUDOMONAS PIGMENT MEDIUM

pancreatic digest of casein (Bacto-Casitone)	0.5%
yeast extract	0.3
agar	0.3
manganese sulfate	.01
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	0.32
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	.08
pH 7.2	

The medium was dispensed in 4 ml. quantities in 13X100 mm. tubes and autoclaved at 121°C for 15 minutes. The medium was stabbed. When the medium showed water soluble pigment it was extracted with chloroform.

MILK AGAR

pancreatic digest of casein (Bacto-Casitone)	0.5%
yeast extract	0.3
agar	1.5
pH 7.0	

The medium was dispensed in 15 ml. quantities in tubes then autoclaved at 121°C for 15 minutes. One ml. of litmus milk was aseptically added to each tube. The medium was then poured into a petri plate. After solidification the medium was inoculated in the center of the dish with a needle for giant colony development. Clearing of the agar about the giant colony was recorded after 24 and 48 hours.

BLOOD AGAR

yeast extract	0.3%
pancreatic digest of casein (Bacto-Casitone)	0.5
sodium chloride	0.5
agar	1.5
pH 7.1	
defibrinated rabbit blood	3.5 ml.

The medium was dispensed in 15 ml. quantities and autoclaved at

121°C for 15 minutes. The liquid medium was cooled to 45°C and defibrinated rabbit blood and proper inoculum added. The poured plates were incubated for 48 hours then refrigerated at 8°C for 12 hours and hemolysis studied with the aid of a 35X lens system. Both deep and surface hemolysis was recorded.

#### LITMUS MILK

Bacto Litmus Milk was used.

The medium was dispensed in 5 ml. amounts in 13X100 mm. tubes and sterilized by steam at 100°C for 45 minutes on three successive days. The medium following inoculation and incubation was observed for changes after 1, 2, 3, 4, 5, 6, 7, 10 and 15 days. The pH after 15 days was determined with the glass electrode.

#### GROWTH ON DESOXYCHOLATE AGAR

Leifson's desoxycholate agar (1935) was used.

pancreatic digest of casein (Bacto-Casitone)	1.0%
agar	1.7
sodium chloride	0.5
dipotassium phosphate	0.2
lactose	1.0
ferric ammonium citrate	0.2
sodium desoxycholate (Baltimore Biol. Lab.)	0.1
neutral red (1% aqueous solution)	0.33 ml.

Two organisms were streaked lightly on each plate. Growth on the medium was recorded after 18-24 hours incubation as it compared with growth on three other concentrations of sodium desoxycholate, namely 0%, 0.2% and 0.5%.

Table I lists the organisms studied giving the source of isolation and donor. The blank under the heading RECEIVED AS indicates no name was

assigned at the initiation of the study. The source of isolation of many strains is probably unknown, hence the blank space under the heading SOURCE.

The following abbreviations were used to indicate the source of the cultures:

ANE	Academy of Natural Sciences, Philadelphia, Pennsylvania.
ATCC	American Type Culture Collection, Washington, D. C.
ISPHL	Illinois State Public Health Laboratories, Chicago, Illinois.
MSH	Mount Sinai Hospital, Chicago, Illinois.
NCTC	National Collection of Type Cultures, London, England.
NIH	National Institute of Health, Bethesda, Maryland.
NRRL	Northern Regional Research Laboratory, Peoria, Illinois.
SSM	Stritch School of Medicine of Loyola University, Chicago, Illinois.

TABLE I  
THE CULTURE COLLECTION

RH #	RECEIVED AS	SOURCE	DONOR
1	<u>Pseudomonas aeruginosa</u>		Univ. Ill. College Med.
2	<u>Alcaligenes strain SF</u>	dipeptide solution	S. Simmonds, Yale Univ.
3	<u>Pseudomonas aeruginosa</u>	well water	R. Hugh, SSM
5	<u>Alcaligenes faecalis</u>		Univ. Ill. College Med.
6		frog tank water	R. Hugh, SSM
7		frog tank water	R. Hugh, SSM
9			F. Friewer, LSPHL 1571B
10	<u>Alcaligenes hemolysans</u>	spinal fluid	P. Oeding, Univ. Bergen, Norway 1058
11	<u>Alcaligenes non-hemolytic</u>	spinal fluid	P. Oeding, Univ. Bergen, Norway
12	<u>Alcaligenes sp.</u>	soil	L. Frederick, Purdue Univ. 55B
13	<u>Alcaligenes sp.</u>	soil	L. Frederick, Purdue Univ. 17B
14	<u>Alcaligenes faecalis</u>		E. Wynne, Univ. Okla.
15	<u>Alcaligenes viscosus</u>		E. Wynne, Univ. Okla.
16	<u>Alcaligenes sp.</u>	stool	M. Fulton, SSM 66
17	<u>Alcaligenes sp.</u>	diarrheal stool	M. Fulton, SSM 539
18		frog tank water	R. Hugh, SSM

19		frog tank water	R. Hugh, SSM
21	<u>Alcaligenes bockeri</u>	beef	J. Edwards, ATCC9128, same as NCTC 6535
22	<u>Alcaligenes faecalis</u> var. <u>radicans</u>	blood	A. Evans, ATCC 4741, see RH 228 and 377
23	<u>Alcaligenes viscosus</u>		B. Hammer, ATCC 9036
24	<u>Alcaligenes</u> sp.		W. Randall, ATCC 10153
25	<u>Alcaligenes</u> sp.	blood	M. Fulton, SSM 1954
26	<u>Alcaligenes</u> sp.	diarrheal stool	M. Fulton, SSM 2255
27	<u>Alcaligenes</u> sp.	diarrheal stool	M. Fulton, SSM 2258
28	<u>Alcaligenes</u> sp.	diarrheal stool	M. Fulton, SSM 2294
29	<u>Alcaligenes</u> sp.	old stock culture	M. Fulton, SSM 2392
31	<u>Alcaligenes</u> sp.	diarrheal stool	M. Fulton, SSM 2445
32	<u>Alcaligenes</u> sp.		M. Fulton, SSM 3046
35	<u>Alcaligenes viscosus</u>		Iowa State College 1
36	<u>Alcaligenes viscosus</u>		Iowa State College 2
37	<u>Alcaligenes</u> sp.	water	M. Fulton, SSM 2686
38		dead frog's heart	R. Hugh, SSM
42	<u>Brucella melitensis</u>		Univ. Ill. College Med.
43	<u>Brucella melitensis</u>		NIH 428
44	<u>Brucella abortus</u>		NIH 456

45	<u>Brucella suis</u>		NIH 483
46	<u>Brucella bronchiseptica</u>	dog	J. Ray, Nebraska 107
47	<u>Brucella bronchiseptica</u>	dog	J. Ray, Nebraska 3465
48	<u>Brucella bronchiseptica</u>	pig	J. Ray, Nebraska 3672
49	<u>Brucella bronchiseptica</u>	pig	J. Ray, Nebraska 4707
50	<u>Brucella bronchiseptica</u>	pig	J. Ray, Nebraska 5126
51	<u>Brucella bronchiseptica</u>	pig	J. Ray, Nebraska 5224
52	<u>Brucella bronchiseptica</u>	dog	J. Ray, Nebraska 5246
53	<u>Brucella bronchiseptica</u>	dog	J. Ray, Nebraska 6025
54	<u>Brucella bronchiseptica</u>	dog	J. Ray, Nebraska 4432
55	<u>Pseudomonas aeruginosa</u>		Chicago Med. College 1
56	<u>Pseudomonas aeruginosa</u>		Chicago Med. College 2
57	<u>Pseudomonas aeruginosa</u>		Chicago Med. College 3
58	<u>Pseudomonas aeruginosa</u>		Chicago Med. College
59	<u>Pseudomonas fluorescens</u>		Chicago Med. College 1
60	<u>Alcaligenes faecalis</u>		Chicago Med. College
61	<u>Pseudomonas fluorescens</u>		Chicago Med. College 2
62	<u>Heliconema</u> sp.	feces of child	E. Olsen, Denmark 1
63	<u>Heliconema</u> sp.	feces of child	E. Olsen, Denmark 2



64	<u>Heliconema</u> sp.	feces of child	E. Olsen, Denmark 3
65	<u>Heliconema</u> sp.	feces of child	E. Olsen, Denmark 4
66	<u>Alcaligenes</u> sp.		M. Fulton, SSM 3130
67	<u>Brucella abortus</u>		S. Garza, Laredo, Texas
68	<u>Brucella abortus</u>		S. Garza, Laredo, Texas
69	<u>Brucella suis</u>		S. Garza, Laredo, Texas
70	<u>Brucella suis</u>		S. Garza, Laredo, Texas
71	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas
83	<u>Alcaligenes faecalis</u>	old stock strain	E. Weinberg, Univ. Chic.
89		Chicago tap water	R. Hugh, SSM
95		Chicago tap water	R. Hugh, SSM
107		Wheaton tap water	R. Hugh, SSM
110	<u>Alcaligenes faecalis</u>	stool	M. Goldin, MSH A
111	<u>Alcaligenes faecalis</u>	stool	M. Goldin, MSH B
112	<u>Pseudomonas aeruginosa</u>		M. Goldin, MSH
113	<u>Pseudomonas aeruginosa</u>		M. Goldin, MSH
114	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas 3021
115	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas 4801
117	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas 5063

118	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas 5251
119	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas 5375
120	<u>Brucella melitensis</u>		S. Garza, Laredo, Texas
122	<u>Pseudomonas aeruginosa</u>		H. Hoffmann, Ohio State Univ. 330
123	<u>Pseudomonas aeruginosa</u>		New York Botanical Gardens "Don"
124	<u>Alcaligenes</u> sp.	stool	M. Fulton, SSM 2120
125	<u>Alcaligenes faecalis</u>	blood	W. Lane, England 1
126	<u>Alcaligenes faecalis</u>	blood	W. Lane, England 2
127			L. Frederick, Purdue Univ. 37B
128		water	M. Goldin, MSH
129		urine	M. Goldin, MSH L864
130		urine	M. Goldin, MSH L834
131		stool	M. Goldin, MSH L444
132		blood	M. Goldin, MSH Guzman
133		stool	M. Goldin, MSH L579
135	<u>Alcaligenes faecalis</u>		H. Conn, ATCC 8750
136	<u>Alcaligenes faecalis</u>	stool	O. Falsenfeld, ATCC 9220
137	<u>Alcaligenes faecalis</u>		E. Jordan, ATCC 212
138	<u>Alcaligenes viscosus</u>	ropy milk	L. Rogers, ATCC 337, same as NCTC 3233

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139	<u>Alcaligenes faecalis</u>	G. Phillips, Camp Detrick, Maryland
140	<u>Brucella bronchiseptica</u>	G. Phillips, Camp Detrick, Maryland B-140
141	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-7, same as Smith's 112 <u>Pseudomonas fluorescens</u>
142	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-12, same as ATCC 142 <u>Pseudomonas fluorescens</u>
143	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-221
144	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-241, same as ATCC 257
145	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-247, same as ATCC 97
146	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-248, same as ATCC 256
147	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-249, same as ATCC 260
148	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-250, same as ATCC 262
149	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-275, same as Stark's 150
150	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL B-996, same as NCTC 5083
151	<u>Pseudomonas aeruginosa</u>	W. Haynes, NRRL 1069, same as NCTC 8028 <u>Mallozyces hamorricogones</u>
153	<u>Pseudomonas</u> sp.	W. Haynes, NRRL B-85
154	<u>Pseudomonas</u> sp.	W. Haynes, NRRL B-90
155	<u>Pseudomonas</u> sp.	W. Haynes, NRRL B-102
156	<u>Pseudomonas</u> sp. ( <u>Aeromonas</u> )	W. Haynes, NRRL B-538

157	<u>Pseudomonas mucidolens</u>		W. Haynes, NRRL B-726
159		stool	R. Hugh, SSM
160		stool	R. Hugh, SSM
161		stool	R. Hugh, SSM
162		catheterized urine	R. Hugh, SSM 4788
163		sputum	R. Hugh, SSM 4795
164	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 351
165	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 413
166	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 414
167	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 415
168	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 434
169	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 4561
170	<u>Alcaligenes hemolyans</u>	throat	S. Henriksen, Norway 479
171	<u>Brucella bronchiseptica</u>	guinea pig lung	S. Henriksen, Norway I
172	<u>Brucella bronchiseptica</u>	guinea pig lung	S. Henriksen, Norway II
173	<u>Brucella bronchiseptica</u>	guinea pig lung	S. Henriksen, Norway III
174	<u>Alcaligenes faecalis</u>		ATCC 8748 "atypical strain" received 3-1-52, see RH 378
175	<u>Alcaligenes faecalis</u>		H. Conn, ATCC 8749 "atypical strain"
176	<u>Agrobacterium radiobacter</u>		A. Hildebrandt, Univ. Wisc.

177	<u>Agrobacterium tumefaciens</u>		A. Hildebrandt, Univ. Wisc. same as Riker's strain A6
180	<u>Brucella bronchiseptica</u>		ATCC 4617, same as NRRL B-140
181	<u>Brucella bronchiseptica</u>	human	ATCC 786
182	<u>Brucella bronchiseptica</u>	murine	ATCC 785
183	<u>Brucella bronchiseptica</u>	canine	ATCC 780
184	<u>Brucella bronchiseptica</u>		ATCC 19
185		stool	F. Friewer, ISPHL 10091SSR <sub>1</sub>
186		stool	F. Friewer, ISPHL 10775A
187		stool	F. Friewer, ISPHL 7332T
188		stool	F. Friewer, ISPHL 7101TT
189		stool	F. Friewer, ISPHL 7332SS
190		stool	F. Friewer, ISPHL 7080T <sub>2</sub>
191		stool	F. Friewer, ISPHL 7198S <sub>2</sub>
192		stool	F. Friewer, ISPHL 7254S
193		stool	F. Friewer, ISPHL 7304SS
194		stool	F. Friewer, ISPHL 11002A
195		stool	F. Friewer, ISPHL 7015AR <sub>2</sub> R <sub>1</sub>
196		stool	F. Friewer, ISPHL 10864
197		stool	F. Friewer, ISPHL 10714R <sub>1</sub>

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198		stool	F. Friewer, ISPHL 7283A
199		stool	F. Friewer, ISPHL 4252 (L)
200		stool	F. Friewer, ISPHL 4252B
201		stool	F. Friewer, ISPHL 11002B <sub>1</sub>
202		stool	F. Friewer, ISPHL 7232T
203		stool	F. Friewer, ISPHL 7370A
204		stool	F. Friewer, ISPHL 10604A
205		stool	F. Friewer, ISPHL 7378A
207		stool	F. Friewer, ISPHL 11231TR <sub>1</sub>
208		stool	F. Friewer, ISPHL 7015BR <sub>1</sub>
209		stool	F. Friewer, ISPHL 7179S <sub>2</sub>
210		blood	F. Friewer, ISPHL 10949BC <sub>1</sub>
211		blood	F. Friewer, ISPHL 10663BC
212		blood	F. Friewer, ISPHL 10958BC <sub>1</sub>
213		blood	F. Friewer, ISPHL 10980BC
214	<u>Haemophilus bronchisepticus</u>	lung of dog	NCTC 452
216	<u>Alkaligenes faecalis</u>	stool	Murray, McGill Univ. P1696
217	<u>Alkaligenes faecalis</u>	nasal swab	Murray, McGill Univ. P2693
218	<u>Alkaligenes faecalis</u>	chest drainage	Murray, McGill Univ. P3325

219	<u>Alkaligenes faecalis</u>	urine	Allin, Fort Williams MB81-51
222	<u>Bacterium alcaligenes</u>	feces	NCTC 415
223	<u>Bacterium alcaligenes</u>		NCTC 655
224	<u>Bacterium alcaligenes</u>	urine	S. Dyke, NCTC 1200
225	<u>Bacterium alcaligenes</u>	guinea pig lung	NCTC 1279
226	<u>Bacterium alcaligenes</u>		NCTC 1347
227	<u>Bacterium alcaligenes</u>	blood	NCTC 1704, same as Rhmen 78
228	<u>Bacterium faecalis alcaligenes</u> <u>var. radicans</u>	blood	A. Evans, NCTC 3512, see RH 22 and 377
229	<u>Bacterium bookeri</u>	pleural fluid?	NCTC 6572
231		egg	F. Friewer, ISPHL 6789A
232	<u>Brucella bronchiseptica</u>	throat of dog	L. LeBeau, Univ. Ill. Col. Med. 11-1
242	<u>Bacterium alcaligenes</u>	otitis media	S. Cowan, England "L"
245	<u>Alcaligenes faecalis</u>		Midwest Culture Service, Terre Haute, Indiana
246	<u>Agrobacterium tumefaciens</u>		Midwest Culture Service, Terre Haute, Indiana
247	<u>Vibrio parcolans</u>	hay infusion	Mudd and Warren, ATCC 8461, see RH 260 and 261
248	<u>Vibrio cuneatus</u>	soil	B. Gray, ATCC 6972
249	<u>Pseudomonas saccharophilia</u>	stagnant water	M. Doudoroff, ATCC 9114

250	<u>Pseudomonas ichthyosmia</u>	spoiled milk	M. Speck, ATCC 7966, same as NCTC 8049
251	<u>Pseudomonas hydrophila</u>		M. Speck, ATCC 7965, same as NCTC 7812
252	<u>Pseudomonas hydrophila</u>	frog septicemia	W. Kulp, ATCC 9071
253	<u>Aeromonas liquefaciens</u>		A. Kluyver, Holland 1417
254	<u>Aeromonas liquefaciens</u>	canal water	A. Kluyver, Holland 1418, see RH 271
255	<u>Aeromonas hydrophila</u>	frog septicemia	A. Kluyver, Holland 1417
257	<u>Alcaligenes</u> sp.	stool	F. Friewer, ISPHL 2733R <sub>1</sub>
258	<u>Alcaligenes</u> sp.	stool	F. Friewer, ISPHL 2733
259	<u>Alcaligenes</u> sp.	otitis media	S. Cowan, England, "p"
260	<u>Vibrio percolans</u>	variant of RH 247	R. Hugh, SSM
261	<u>Vibrio percolans</u>	variant of RH 247	R. Hugh, SSM
262	<u>Acetobacter aceti</u>		ATCC 8303
263	<u>Acetobacter oxydans</u>		ATCC 9433
266	<u>Pseudomonas</u> sp.	paracentesis fluid	F. Friewer, ISPHL 3369
268		stream	Keller, ANS 493 11/2
269		stream	Keller, ANS 517 11/4
271	<u>Aeromonas liquefaciens</u>	canal water	E. Miles, England 1418, see RH 254
272	<u>Chromobacterium amethystinum</u>		Hopkins Marine Sta., Calif., M. O. 2.1
273	<u>Spirillum virginianum</u>		Hopkins Marine Sta., Calif., O.1.1



274	<u>Rhodospirillum rubrum</u>	Hopkins Marine Sta., Calif., ATR. 1.1.1
275	<u>Rhodopseudomonas palustris</u>	Hopkins Marine Sta., Calif., ATR. 2.1.1
277	<u>Acetobacter rancens</u>	Hopkins Marine Sta., Calif., M. A. 7.1
280	stream	Keller, ANS 50 L1/3
283	stream	Keller, ANS 72 L1/2
284	stream	Keller, ANS 100 L1/2
287	stream	Keller, ANS 154 L1/2
289	stream	Keller, ANS 156 L1/3
292	stream	Keller, ANS 169 L1/3
294	stream	Keller, ANS 186 L1/2
295	stream	Keller, ANS 196 L1/2
296	stream	Keller, ANS 199 L1/2
298	stream	Keller, ANS 220 L1/2
300	stream	Keller, ANS 225 L1/2
302	stream	Keller, ANS 229 L1/3
304	stream	Keller, ANS 234 L1/2
306	stream	Keller, ANS 239 L1/2
307	stream	Keller, ANS 247 L1/2
308	stream	Keller, ANS 255 L1/2

309	stream	Keller, ANS 262 L1/2
310	stream	Keller, ANS 265 L1/2
311	stream	Keller, ANS 269 L1/2
312	stream	Keller, ANS 270 L1/2
313	stream	Keller, ANS 281 L1/2
314	stream	Keller, ANS 286 L1/2
318	stream	Keller, ANS 309 L1/2
320	stream	Keller, ANS 328 L1/2
322	stream	Keller, ANS 332 L1/2
324	stream	Keller, ANS 334 L1/2
330	stream	Keller, ANS 339 L1/2
333	stream	Keller, ANS 340 L1/2
335	stream	Keller, ANS 341 L1/2
336	stream	Keller, ANS 360 L1/2
338	stream	Keller, ANS 361 L1/2
339	stream	Keller, ANS 362 L1/4
341	stream	Keller, ANS 366 L1/2
342	stream	Keller, ANS 367 L1/2
343	stream	Keller, ANS 408 L1/2

344	stream	Keller, ANS 410 LI/2
347	stream	Keller, ANS 433 LI/2
349	stream	Keller, ANS 434 LI/2
353	stream	Keller, ANS 473 LI/2
355	stream	Keller, ANS 482 LI/2
360	blood of frog with red leg	R. Hugh, SSM
363	blood of frog with red leg	R. Hugh, SSM
364	blood of frog with red leg	R. Hugh, SSM
370	parapertussis bacillus	G. Eldering, Mich. Dept. Health 21-353
373	parapertussis bacillus	G. Eldering, Mich. Dept. Health 21-656
374	parapertussis bacillus	G. Eldering, Mich. Dept. Health 23-456
375	<u>Pfeifferella pseudomallei</u>	Guy, NCTC 7431
376	<u>Alcaligenes</u> sp.	ATCC 213, received thru J. Ulrich of Mayo Clinic, Minnesota
377	<u>Alcaligenes</u> sp.      blood	A. Evans, ATCC 4741, received thru J. Ulrich of Mayo Clinic, Minnesota, see RH 22 and 228
378	<u>Alcaligenes faecalis</u>	ATCC 8748, received thru J. Ulrich of Mayo Clinic, Minnesota, see RH 174

379	<u>Alcaligenes</u> sp.	feces	ATCC 9220, received thru J. Ulrich of Mayo Clinic, see RH 136
380	<u>Alcaligenes</u> sp.	old stock culture	J. Ulrich, Mayo Clinic U10
383		orange	E. Leifson, SSM
384	<u>Pseudomonas</u> sp.	dysentery stool	M. Fulton, SSM 3108
385	<u>Alcaligenes</u> sp.	abdominal swab	M. Fulton, SSM 2781
387	<u>Salmonella</u> <u>typhosa</u>		Panama 58 strain
388	<u>Salmonella</u> <u>paratyphi</u>		Univ. Ill. College Med.
389	<u>Salmonella</u> <u>schottmuelleri</u>		O. Felsenfeld, Hektoen Inst., Chicago
390	<u>Salmonella</u> <u>enteriditis</u>		O. Felsenfeld, Hektoen Inst., Chicago
398	<u>Proteus</u> <u>morganii</u>		M. Fulton, SSM 2181
401	<u>Proteus</u> <u>rettgeri</u>		M. Fulton, SSM 3457
405	<u>Proteus</u> <u>vulgaris</u>		M. Fulton, SSM 3488
406	<u>Escherichia</u> <u>coli</u>		Univ. Ill. College Med.
407	<u>Aerobacter</u> <u>aerogenes</u>		Univ. Ill. College Med.
409	<u>Vibrio</u> <u>paracholera</u>		O. Felsenfeld, Hektoen Inst., Chic.
410	<u>Bacterium</u> <u>anitratum</u>		W. Ferguson, Mich. Dept. Health 5W2
411	<u>Bacterium</u> <u>anitratum</u>		W. Ferguson, Mich. Dept. Health 5W3
412	<u>Vibrio</u> <u>comma</u> <u>ogawa</u>		O. Felsenfeld, Hektoen Inst. Chic.
413	<u>Vibrio</u> <u>comma</u> <u>inaba</u>		O. Felsenfeld, Hektoen Inst. Chic.

Table I continued from page 90

414	<u>Shigella paradysenteriae</u> IV		
417	<u>Pseudomonas</u> sp.		M. Fulton, SSM 2681
418	<u>Pseudomonas</u> sp.	urine	M. Fulton, SSM 2689
419	<u>Pseudomonas</u> sp.	urine	M. Fulton, SSM 2803
420	<u>Pseudomonas</u> sp.		M. Fulton, SSM 2805
421	<u>Pseudomonas</u> sp.		M. Fulton, SSM 2820
422	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3051
423	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3099
424	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3100
425	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3123
426	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3124
427	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3140
428	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3154
429	<u>Pseudomonas</u> sp.		M. Fulton, SSM 3162
430	<u>Alcaligenes</u> sp.		J. Ulrich, Mayo Clinic 249
431	<u>Alcaligenes</u> sp.		J. Ulrich, Mayo Clinic 282
432	anaerogenic paracolon bacillus	<u>Endamoeba histol.</u> WRS culture	W. Balamuth, Northwestern Univ.
433	paracolon bacillus	stool	F. Friewer, ISPHL 6630T
436	<u>Shigella madampensis</u>	old stock culture	F. Friewer, ISPHL 229

Table I continued from page 90

437	<u>Shigella sonnei</u> ph I	old stock culture	F. Friewer, ISPHL 247
439	<u>Spirillum rubrum</u>		O. Felsenfeld, Hektoen Inst., Chicago
443		chalk stream	E. Gray, England G <sub>2</sub>
444		chalk stream	E. Gray, England G <sub>4</sub>

CHAPTER V

EXPERIMENTAL DATA

Photomicrographs Showing Bacterial Flagella



Figure 1

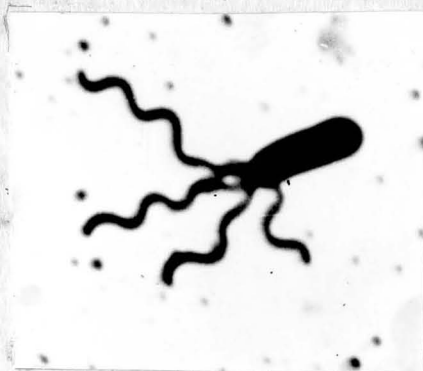


Figure 2

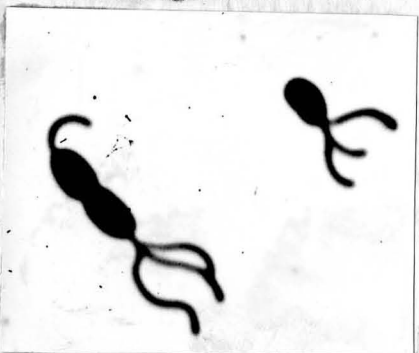


Figure 3

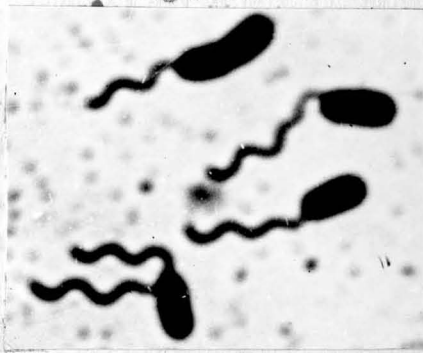


Figure 4



Figure 5

- Figure 1. Alcaligenes bronchicanis showing typical peritrichous flagellation. Photomicrograph X4000.
- Figure 2. Xanthomonas maltophilia sp. nov. showing typical polar multitrichous flagellation. Photomicrograph X4000.
- Figure 3. Lophobacter faecalis gen. et sp. nov. showing typical lophotrichous flagellation. Photomicrograph X4000.
- Figure 4. Pseudomonas aeruginosa showing typical monotrichous flagellation. Photomicrograph X4000.
- Figure 5. Pseudomonas sp. showing "curly" monotrichous flagellation. See Table XIV RH numbers 339, 342 and 347. Photomicrograph X4000.

## KEY TO THE TABLES

peri	peritrichous
polar multi	polar multitrichous
lopho	lophotrichous
mono	monotrichous
?	type of polar flagellation may be questioned.
-	nonmotile, negative test, no hemolysis
+	positive test or turbidity
2+	degree of turbidity greater than +
@	positive test for nitrite and gas, acid and gas
±	poor growth
blank	test not performed
A	alpha hemolysis
B	beta hemolysis
b	blue color of litmus
r	red color of litmus
nc	no change of indicator
f	carbohydrate fermented



TABLE II

## CHARACTERISTICS OF THE ORGANISMS

																1% casitone	pigment			blood agar hemol.	litmus milk ,						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color pH	desoxycholate	
1	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	-	+	2+	+	-		+	-	+	b 8.3	+
2	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-		-	-	-	b	+
3	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	-	+	2+	+	+		+	-	+	b 8.4	+
5	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	+		-	-	-	-	b 8.0	+
6	?mono	-	-	-	-	-	-	-	-			+	-	-	-	-	-	+	-	-		-	-	-	-	b 8.1	-
7	?polar multi	nc	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-		+	-	-	-	b 8.1	±
9	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-		-	A	+	+	b 7.8	+
10	-	+	+	-	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-		-	B	+	+	r	+
11	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-		-	-	-	-	r	+
12	peri	-	-	-	-	-	-	-	-	-	-	+	-	-	-	⊖	-	+	2+	-		-	-	-	-	b 8.4	+
13	peri	-	-	-	-	-	-	-	-	-	-	+	-	-	-	⊖	-	+	2+	-		-	-	-	-	b 8.5	+
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-		-	-	-	-	nc	-





Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment		blood agar hemol.	litmus milk							
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep	surface	color pH	desoxycholate
54	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-			-	-	-	b 8.6	+
55	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-		+	-	+	b 8.0	+
56	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-		-	-	+	b 7.9	+
57	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+		+	-	+	b 7.9	+
58	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-		+	-	+	b 7.9	+
59	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-		+	-	+	b 8.1	+
60	peri	nc	-	-	-	-	+	-	-	-	-	+	-	-	-	⊖	-	+	2+	-			-	-	-	b 8.5	+
61	-	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	-	+	2+	+			+	-	+	b 8.2	+
62	-	nc	nc	nc	nc	nc	nc	nc	-	-	-	-	-	-	-	-	-	+	-	-			-	-	-	r	-
63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-			-	-	-	nc	-
64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-			-	-	-	nc	-
65	-	nc	nc	nc	nc	nc	nc	nc	-	-	-	-	-	-	-	-	-	+	-	-			-	-	-	b	-
66	mono	+	-	-	+	+	+	-	-	-	-	+	-	-	-	⊖	-	+	2+	-			-	-	+	b 8.3	+
83	peri	nc	-	-	-	-	+	-	-	-	-	+	-	-	-	⊖	-	+	2+	-			-	-	-	b 8.4	+

Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment			blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep	surface	color pH	desoxycholate
89	mono	+	-	-	-	-	-	-	-	-	-	-	-	-	-	⊖	-	+	2+	-	-	-	-	-	-	b 6.9	-
95	mono	+	-	-	-	-	-	-	-	-	-	-	-	-	-	⊖	-	+	2+	-	-	-	-	-	-	b 7.0	-
107	mono	nc	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	b 8.2	-
110	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	A	+	b 7.7	+
111	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+	+	+	-	+	b 8.0	+
112	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+	+	+	-	+	b 8.0	+
113	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+	+	+	-	+	b 8.1	+
122	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	-	+	2+	+	+	+	+	-	+	b 7.7	+
123	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+	+	+	-	+	b 8.0	+
124	lopho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	b 7.9	+
125	mono	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	b 7.0	+
126	mono	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	b 7.1	+
127	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	b 8.0	-
128	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	A	+	b 8.0	+

Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment			blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methy1 red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color pH	desoxycholate	
129	?mono	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	b 8.2	-
130	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	r	+
131	lopho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	2+	+	-	-	-	-	-	-	b 6.7	+
132	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	A	+	b 8.0	+
133	lopho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	2+	+	-	-	-	-	-	-	b 8.1	+
135	peri	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	A	+	b 8.4	+
136	peri	nc	-	-	-	-	+	-	-	-	-	+	-	-	-	⊖	-	+	2+	-	-	-	-	-	-	b 8.4	+
137	peri	nc	-	-	-	-	+	-	-	-	-	+	-	-	-	⊖	-	+	2+	-	-	-	-	-	-	b 8.4	+
138	peri	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	b 8.4	±
139	-	nc	nc	nc	nc	nc	nc	nc	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	nc	-
140	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	-	-	-	b 8.5	+
141	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-	-	+	-	+	b 7.7	+
142	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-	-	+	-	+	b 7.9	+
143	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	-	-	+	-	+	b 7.8	+

Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment			blood agar hemol.	litmus milk		
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum no petrolatum NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble CHCl <sub>3</sub> soluble color	milk agar	deep surface	color pH	desoxycholate	
144	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.4	+
145	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.3	+
146	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	-	+	-	+	b 8.1	+
147	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.2	+
148	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.1	+
149	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	- + 2+	+	+	-	+	b 7.6	+
150	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.0	+
151	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.2	+
153	polarmulti	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	+	-	-	-	b	+
154	?polar multi	+	+	-	+	+	+	+	-	-	-	+	-	-	-	-	- + -	+	-	-	-	r	+
155	mono	+	+	-	+	+	+	+	-	-	-	+	-	-	-	-	- + -	-	-	-	-	b 6.4	+
156	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	-	+	b 8.1	+
157	mono	+	-	-	-	+	+	+	-	-	-	-	-	-	-	⊖	- + -	-	-	-	-	b 7.7	+
159	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	-	-	-	-	r	-





Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment			blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep	surface	color pH	desoxycholate
174		fb																									
175	-	nc	nc	nc	nc	nc	nc	nc	-	-	-	-	-	-	-	-	-	+	-	-	-		-	-	-	nc	-
176	peri	+	-	+	+	+	+	+	-	-	-	-	+	-	-	+	-	+	-	-	-		-	-	-	nc	-
177	peri	+	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-		-	-	-	b 7.7	-
180	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-		-	-	-	b 8.3	+
181	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-		-	-	-	b 8.5	+
182	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-		-	-	-	b 8.5	+
183	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-		-	-	-	b 8.5	+
184	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-		-	-	-	b 8.6	+
185	lopho	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	2+	+	-	-		-	-	-	b 8.4	+
186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-		-	B	+	b 8.0	+
187	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+		+	-	-	b 7.6	+
188	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	-	+	2+	+	+		+	A	-	b 8.3	+
189	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	-	+	2+	+	+		+	-	-	b 7.7	+

Table II contd. from page 109

RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	1% casitone	pigment	blood agar hemol.	litmus milk	
																	petrolatum no petrolatum NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble CHCl <sub>3</sub> soluble color	milk agar deep surface	color pH	desoxycholate
190	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊖	- + 2+	+	+	-	+
191	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	+	b 8.3
192	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	+	b 8.2
193	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	+	-	-	b
194	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	- + -	-	B	+	b 8.1
195	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	-	+	b 8.2
196	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	+	+	b
197	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	+	-	B	b
198	mono	+	-	-	+	-	-	-	-	-	-	+	-	-	-	+	- + -	-	-	-	b 8.3
199	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	-	-	-	b 8.2
200	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	- + -	-	-	-	b 8.3
201	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	- + -	+	brown	-	b 8.0
202	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊖	- + 2+	+	-	+	b 8.3
203	mono	+	-	-	+	+	+	-	-	-	-	+	-	-	-	+	- + -	-	-	-	b

Table II contd. from page 109

Table II contd. from page 109																	1% casitone	pigment		blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep	surface	color pH	desoxycholate
204	mono	+	-	-	+	+	+	-	-			+	-	-	-	⊕	-	+	2+	-			-	-	-	b 8.3	+
205	polar multi	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	2+	+	-	yellow		-	-	-	b 8.0	-
207	?lopho	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊕	-	+	+	-	yellow		-	-	-	b 8.1	+
208	mono	+	-	-	-	+	+	+	-	-	-	+	-	-	-	⊕	-	+	2+	+	-		+	-	+	b 7.5	+
209	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊕	-	+	+	+	-		+	-	+	b 7.5	+
210	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊕	-	+	+	+	-		+	B	+	b 8.4	+
211	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-			-	-	-	b 8.0	+
212	?lopho	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-			-	-	-	b 6.9	+
213	polar multi	+	-	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	+	-		-	-	-	b	+
214	peri	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-			-	-	-	b 8.6	±
216	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-			-	B	+	b 7.7	+
217	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊕	-	+	+	+	+		+	-	+	b 7.7	+
218	mono	+	-	-	-	+	+	+	-	-	-	+	-	+	-	⊕	-	+	2+	+	+		+	-	+	b 7.8	+
219	-	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-			-	-	-	b 7.9	+

Table II contd. from page 109

Table II contd. from page 109															1% casitone		pigment		blood agar hemol.		litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color	pH	desoxycholate
222	peri	-	-	-	-	-	-	-	-	-		+	-	+	-	-	-	+	-	-			-	-	-	b 8.5	+
223	peri	-	-	-	-	-	-	-	-	-		+	-	-	-	-	-	+	-	-			-	A	+	b 8.2	+
224		f0																									
225	peri	-	-	-	-	-	-	-	-	-		-	+	-	-	+	-	+	-	-			-	-	-	b 8.6	-
226	lopho	-	-	-	-	-	-	-	-	-		-	-	-	-	+	-	2+	+	-			-	-	-	b 8.6	+
227	peri	-	-	-	-	-	-	-	-	-		-	+	-	-	+	-	+	-	-			-	-	-	b 8.5	-
228		f0																									
229	polar multi	-	-	-	+	-	-	-	-	-		-	-	+	-	+	-	2+	+	-			+	-	-	b 8.0	+
231	mono	-	-	-	-	-	-	+	-	-		-	-	+	+	+	-	2+	+	-	pink orange		-	A	-	b 8.3	+
232	peri	-	-	-	-	-	-	-	-	-		+	+	-	-	+	-	+	-	-			-	-	-	b 8.7	+
242	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	0	-	+	2+	-			-			b 8.0	
245	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	+	-	-							
246	peri	+	-	+	+	+	+	+	-	-		-	-	-	-	+				-			-			b 7.1	
247	lopho & peri	-	-	-	-	-	-	-	-	-		-	-	-	-	+	-	+	-	-						b 7.0	

Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment			blood agar hemol.	litmus milk							
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep	surface	color pH	desoxycholate	
248	polar multi	+	-	+	-	+	+	+	-	-		+	-	+	-	+	-	+	-	+				-			b 8.1	
249	mono	-	-	+	-	+	-	-	-	-		-	-	-		⊕	-	+	-	-	yellow- brown		-				nc 6.5	
250	mono	fθ	-																									
251	mono	fθ	-																									
252	mono	fθ	-																									
253	mono	fθ	-																									
254	peri	fθ	-																									
255	mono	fθ	-																									
257	polar multi	+	-	-	-	+	+	+	-	-		+	-	-	-	-	-	+	-	-			-				b 8.4	
258	peri	-	-	-	-	-	-	-	-	-		+	-	-	-	-	-	+	-	-			-				b 8.4	
259	?mono	+	+	-	-	+	+	+	-	-		+	+	-	-	-	-	+	-	-			-				b 8.0	
260	peri	-	-	-	-	-	-	-	-	-		-	-	-	-	+	-	+	-	-							b 6.9	
261	lopho	-	-	-	-	-	-	-	-	-		-	-	-	-	+	-	+	-	-							b 6.9	
262	peri	+	-	-	-	+	+	+	-	-		-	-	-			-	+	-	-							nc 6.6	

Table II contd. from page 109

Table II contd. from page 109																	1% casitone	pigment			blood agar hemol.	litmus milk					
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color	pH	desoxycholate
263	-	+	-	-	-	-	+	-	-	-		-		-			-	+	-	-						b 7.0	
266	mono	+	+	-	+	+	+	+	-	-		+	-	+	-	⊖	-	+	2+	-			-			b 7.2	
268	mono & peri	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-	-			+			b 7.1	
269	mono & peri	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-	-		yellow-orange	+			b 6.7	
271	peri	f⊖	-																								
272	mono	+	-	+	+	+	+	-	-	-		+	-	-		+	-	+	+	-			-			b 7.5	
273	lopho	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-	-			+			nc 7.1	
274	lopho	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	+	-	-		red	-			nc 6.5	
275	mono	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	+	-	-		red	-			b 6.9	
277	-	+	-	-	-	-	+	-	-	-		-	-	-			-	+	-	-			-				
280	polar multi	+	-	-	-	+	+	+	-	-		+	-	-	-	-	-	+	-	+			-			b 8.2	
283	peri	+	-	+	+	+	+	+	-	-		-	+	-	-	-	-			-			-			b 7.8	
284	mono	+	+	-	+	+	+	+	-	-		+	+	-	-	-	-	+	-	-			-			b 5.7	
287	polar multi	-	-	-	+	+	-	-	-	-		-	-	+	-	-	-	+	-	-		yellow	+			b 8.3	

Table II contd. from page 109

Table II contd. from page 109															1% casitone	pigment			blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color pH	desoxycholate
289	mono	f <sup>+</sup>	-																							
292	mono	f <sup>+</sup>	-																							
294	polar multi	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-			+			b 8.3
295	mono	+	+	-	+	+	+	+	-	-		+	-	-	-	-	-	+	-	-			-			b 7.0
296	mono	+	+	-	+	+	+	+	-	-		+	-	-	-	-	-	+	-	-			-			b 7.5
298	polar multi	+	-	-	-	+	+	+	-	-		+	-	-	-	-	-	+	-	+			-			b 8.3
300	mono	+	-	-	-	-	-	-	-	-		+	-	+	-	-	-	+	-	+			+			b 8.0
302	polar multi	+	-	-	-	+	+	+	-	-		+	-	-	-	-	-	+	-	+			-			b 8.2
304	peri	f <sup>+</sup>	+																							
306	mono	f <sup>+</sup>	-																							
307	mono	+	+	+	+	+	+	+	-	-		+	-	-	-	-				-			-			b 7.3
308	mono	+	+	-	+	+	+	+	-	-		+	+	-	-	-	-	+	-	-			-			b 7.2
309	mono	+	+	-	+	+	+	+	-	-		+	+	-	-	-	-	+	-	-			-			b 7.0
310	mono	+	+	-	+	+	+	+	-	-		+	+	-	-	-	-	+	-	-			-			b 7.5

Table II contd. from page 109

Table II contd. from page 109															1% casitone	pigment		blood agar hemol.		litmus milk				
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum no petrolatum NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble CHCl <sub>3</sub> soluble color	milk agar	deep surface	color pH	desoxycholate		
311	mono	+	+	-	+	+	+	+	-	-		+	+	-	-	-	-	+	-			b 7.4		
312	mono	+	+	-	+	+	+	+	-	-		+	-	-	-	-	-	+	-			b 7.4		
313	mono	+	-	-	-	-	-	-	-	-		+	-	-	-	-	+ 2+	+	+			b 7.6		
314	mono	+	-	-	-	-	-	-	-	-		+	-	-	-	-	+ 2+	+	+			b 8.0		
318	peri	+	-	+	+	+	-	-	-	-		-	-	+	-	-	+ 2+	+	-	yellow	+		b 6.5	
320	mono	fθ	-																					
322	peri	-	-	-	-	-	-	-	-	-		-	-	+	-	-	+ 2+	+	-	yellow	+		b 7.5	
324	mono	f+	-																					
330	?mono	-	-	-	-	-	-	-	-	-		-	-	-	0	-	+	+	-	-			nc 6.8	
333	mono	f+	-																					
335	mono	fθ	-																					
336	peri	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-			nc 6.8		
338	peri	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-			nc 6.7		
339	mono	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-			b 8.1		



Table II contd. from page 109

Table II contd. from page 109															1% casitone	pigment			blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum	no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color pH	desoxycholate
341	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	-	-	+	-	+			+		b 7.8	
342	mono	-	-	-	-	-	-	-	-	-		-	-	+	-	-	+	2+	+	-			-		b 7.9	
343	mono	fθ	-																							
344	peri	fθ	+																							
347	mono	-	-	-	-	-	-	-	-	-		-	-	+	-	-	-	+	-	-			-		b 8.2	
249	mono	fθ	-																							
353	mono	fθ	-																							
355	?polar multi	+	-	-	-	+	+	+	-	-		+	-	-	-	-	-	+	-	+			-			
360	-	+	-	-	+	+	-	-	-	-		-	-	-		-	-	+	-	-	yellow					
363	-	+	-	+	-	+	+	+	-	-		+	-	-	-	-	-	+	-	+						
364	mono	+	-	-	+	-	-	-	-	-		-	-	+	+	-	-	2+	+	-	flesh colored					
370	-	-	-	-	-	-	-	-	-	-		-	+	-	-	-	-	+	-	+	brown					
373	-	-	-	-	-	-	-	-	-	-		-	+	-	-	-	-	+	-	+	brown					
374	-	-	-	-	-	-	-	-	-	-		-	+	-	-	-	-	+	-	+	brown					



Table II contd. from page 109

Table II contd. from page 109																1% casitone	pigment		blood agar hemol.	litmus milk						
RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	NO <sub>3</sub>	petrolatum, no petrolatum	NO <sub>3</sub> & petrolatum	H <sub>2</sub> O soluble	CHCl <sub>3</sub> soluble	color	milk agar	deep surface	color	pH	desoxycholate
401	peri	f+	-																							
405	peri	f⊖	-																							
406	peri	f⊖	f⊖																							
407	-	f⊖	f⊖																							
409	mono	f+	-																							
410	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-						
411	-	+	+	-	-	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-						
412	mono	f+	f+																							
413	mono	f+	f+																							
414	-	f+	-																							
417	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	⊖	-	+	2+	+	+					
418	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	⊖	-	+	2+	+	+					
419	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	⊖	-	+	2+	+	-					
420	mono	+	-	-	-	+	+	+	-	-		+	-	+	-	⊖	-	+	-	+	-					



RH number	437 -	439 loph	443 mono	444 filamentous mono
flagellation				
dextrose	+	+	+	+
lactose	+	+	+	+
sucrose	+	+	+	+
maltose	+	+	+	+
mannose	+	+	+	+
xylose	+	+	+	+
arabinose	+	+	+	+
indol	+	+	+	+
methyl red	+	+	+	+
Voges-Proskauer	+	+	+	+
citrate	+	+	+	+
urea	+	+	+	+
gelatin	+	+	+	+
H <sub>2</sub> S	+	+	+	+
NO <sub>3</sub>	+	+	+	+
petrolatum	+	+	+	+
no petrolatum	+	+	+	+
NO <sub>3</sub> & petrolatum	+	+	+	+
H <sub>2</sub> O soluble	+	+	+	+
CHCl <sub>3</sub> soluble	+	+	+	+
color	+	+	+	+
color	+	+	+	+
milk agar	+	+	+	+
deep	+	+	+	+
surface	+	+	+	+
color	+	+	+	+
pH	+	+	+	+
desoxycholate	+	+	+	+

TABLE III

GROUP DIFFERENTIATION OF THE ORGANISMS IN TABLE II




unit	subunit	group	pigm't								RH numbers
			dextrose	lactose	sucrose	flagellation	nitrate	urea	gelatin	H <sub>2</sub> O soluble	
A	1	-	-	-		-	-	-	-	-	135, 223, 258, 376, 378
		-	-	-		-	-	+	-	-	222, 336, 338
		-	-	-		-	-	+	-	+	322 yellow
		-	-	-		-	+	-	-	-	48, 49, 50, 51, 183, 184, 214
		-	-	-		+	-	-	-	-	138, 260
		-	-	-		+	+	-	-	-	29, 46, 47, 52, 53, 54, 140, 171, 172, 173, 180, 181, 182, 225, 227, 232
		-	-	-		⊙	-	-	-	-	12, 13, 60, 83, 136, 137, 379, 380
	2	-	-	-		-	-	+	-	-	294
		-	-	-		-	-	+	-	+	162, 287, 430, 431 yellow
		nc	-	-		+	-	-	-	-	7
		-	-	-		+	-	+	-	-	229
		-	-	-		+	-	+	-	+	205 yellow
	3	-	-	-		-	-	-	-	+	274, 439 red
		-	-	-		-	-	+	-	-	273
		-	-	-		+	-	-	-	-	17, 31, 32, 37, 124, 131, 133, 212, 226, 261, 385
		-	-	-		+	+	-	-	-	185

Table III contd.  
from page 130

pigm't.

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unit	subunit	group	dextrose	lactose	sucrose	flagellation	nitrate	urea	gelatin	H <sub>2</sub> O soluble	H <sub>2</sub> O insoluble	RH numbers
4	-	-	-	-	-	-	-	-	-	-	-	6
	-	-	-	-	-	-	-	-	+	-	-	339, 342, 347
	-	-	-	-	-	-	-	-	+	-	+	38 rose-black
	-	-	-	-	-	-	+	-	-	-	-	21, 27, 28, 125, 126
	-	-	-	-	-	-	-	-	-	-	+	275 red
	-	-	-	-	-		+	-	-	+	-	25, 26 brown
	-	-	-	-	-	-	0	-	-	-	-	330
	-	-	-	-	-	-	+	-	+	-	+	231 pink-orange
	-	-	-	-	-	-	+	+	-	-	-	22
	-	-	+	-	-	-	⊙	-	-	-	+	249 yellow-brown
5	-	-	-	-	-	-	-	-	-	-	-	9, 14, 18, 19, 23, 35, 36, 62, 63, 64, 65, 110, 127, 128, 132, 139, 160, 175, 186, 194, 211, 245
	-	-	-	-	-	-	-	-	-	+	-	201 brown
	-	-	-	-	-	0	-	-	+	-	-	164, 165, 166, 167, 169, 170, 216
	-	-	-	-	-	-	-	+	-	-	-	15
	-	-	-	-	-	-	-	+	-	+	-	370, 373, 374 brown
	-	-	+	-	-	-	-	-	-	-	-	107
	-	-	-	-	-		-	-	+	-	-	268
	-	-	-	-	-		-	-	+	-	+	269 yellow-organge
	-	-	-	-	-		+	-	-	-	-	247

Table III contd.  
from page 130

pigm't.

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unit	subunit	group	dextrose	lactose	sucrose	flagellation	nitrate	urea	gelatin	H <sub>2</sub> O soluble	H <sub>2</sub> O insoluble	RH numbers
B	I	1	+	-	-				-	-	-	262
		2	+	-	-		-	-	-	+	-	5, 153, 193, 197, 213, 280, 298, 302, 355, 384
			+	-	-		-	-	-	-	-	199, 200, 257
		3	+	-	-	Ø	⊕	-	-	-	+	207 yellow
		4	+	-	-		-	-	-	-	-	163
			+	-	-		+	-	-	-	-	203, 198
			+	-	-		⊕	-	-	-	-	66, 89, 95
			+	-	-		-	-	+	-	-	129
			+	-	-		-	-	+	-	+	364 flesh colored
			+	-	-		-	-	-	+	-	2, 313, 314
			+	-	-		-	-	+	+	-	300, 341
			+	-	-		⊕	-	-	-	-	157, 204, 429
			+	-	-		⊕	-	-	+	-	55, 56, 57, 58, 59, 111, 112, 113, 123, 141, 142, 143, 149, 187, 189, 190, 208
			+	-	-		⊕	-	+	+	-	1, 3, 122, 144, 145, 147, 148, 150, 151, 156, 161, 188, 191, 192, 195, 196, 202, 209, 210, 217, 218, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428
			+	-	-		⊕	-	+	-	-	146
			+	-	-		⊕	-	+	-	-	242



Table III contd.  
from page 130

pigm't

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unit	subunit	group	dextrose	lactose	sucrose	flagellation	nitrate	urea	gelatin	H <sub>2</sub> O soluble	H <sub>2</sub> O insoluble	RH numbers
		5	+	-	-		-	-	-	-	-	219
			+	-	-		-	-	-	-	+	360 yellow
			+	-	-	0			-	-		263, 277
			+	-	-		+	-	-	-	-	377
			+	-	-		⊙	-	+	+	-	61
II	1		+	-	+		-	-	+	-	+	318 yellow
			+	-	+		-	+	-	-	-	283
			+	-	+		+	-	-	-	-	246
			+	-	+		-	-	-	-	-	177
			+	-	+		+	+	-	-	-	176
	2		+	-	+		+	-	+	+	-	248
	3		+	-	+		+	-	-	-	-	443, 444, 272
	4		+	-	+	0	-	-	-	+	-	363
III	1		+	+	-		-	-	-	+	-	154
	2		+	+	-		-	-	-	-	-	155, 295, 296, 312
			+	+	-		-	+	-	-	-	284, 308, 309, 310, 311, 259
			+	+	-		⊙	-	+	-	-	266
	3		+	+	-		-	-	-	-	-	11, 16, 24, 130, 159, 410, 411, 432
			+	+	-	0	-	-	+	-	-	168
			+	+	-		-	+	-	-	-	10
IV	1		+	+	+		⊙	-	+	-	-	375
	2		+	+	+		-	-	-	-	-	307



## CHAPTER VI

### DISCUSSION AND CONCLUSIONS

Of the various characteristics which have been used in bacterial taxonomy some are undoubtedly more basically important than others. No single characteristic seems invariable but some appear more variable or unstable than others. Practical considerations have largely dictated the characteristics which are chosen to separate bacteria into various groups. The enteric group is separated by their action on lactose because this at one time seemed to correlate with the prevailing idea of what constituted a pathogen or nonpathogen. The discovery of pathogenic lactose fermenters has tended to minimize the value of this reaction. The slow acceptance of pathogenic nonlactose fermenting bacilli is largely due to the belief that all lactose fermenters were nonpathogenic. Bacteriologists now generally agree that pathogenicity is not a stable characteristic and should occupy a less dominant role in taxonomic systems. Bacteriologists must dissociate themselves from the dictates of specialized groups such as medicine, industry and agriculture and build a taxonomic system based upon biological affinities.

An attempt has been made here to evaluate the taxonomic importance of various characteristics. The present discussion is concerned with gram negative rods which are able to grow in simple peptone or yeast extract in the

presence of air.

### CARBOHYDRATE UTILIZATION

Many types of media have been devised and are in use for the detection of residual acidity from the metabolism of carbohydrates. The usual basal medium for the Enterobacteriaceae consists of 1% peptone, 0.5% sodium chloride, with or without 0.1 to 0.3% beef extract. Sometimes yeast extract is included in the formulae for the more fastidious organisms. Various indicators have been employed. Litmus or Andrade's indicator was used during early times. Now one encounters more frequently a sulphonphalein indicator such as bromcresol purple, phenol red and bromthymol blue. Litmus and Andrade's indicators have not withstood the test of time for they do not give accurate results in terms of hydrogen ion concentration.

Some pseudomonads produce a transient surface change of indicator to the acid color in some of the above media. However, most media now in use show no such changes or, at best, only very slight transient changes of reaction.

Many reports in the literature, as well as the author's frequent observation, indicates that the composition of the basal medium containing organic nitrogen may greatly influence the results obtained in a study of carbohydrate degradation by organisms concerned here.

Pseudomonas pyocyanea, studied by DeBord (1923) in a medium containing 2% peptone, M/20 phosphate and 1% glucose, utilized all the sugar but did not produce an acid reaction. Sears and Courley (1928) found that when Pseudomonas aeruginosa was grown in 0.1 to 5% peptone water, it brought about

a pH of 8.8 in 6 days at which point the reaction remained stationery. These authors found, as did Elrod and Braun (1942), that the nitrogen metabolism resulted in the accumulation of alkaline substances sufficient to keep the acid products of sugar metabolism neutralized. This was not true in media containing small quantities of peptone, for here, a marked rise in acidity resulted.

Cone (1939) using a medium with 0.5% peptone found Pseudomonas produced acid end products from various carbohydrates. Salvin and Lewis (1946) studied what they called fermentation of Pseudomonas on an organic nitrogen medium (peptone 1%, beef extract 0.1%, sodium chloride 0.5% with phenol red indicator) in comparison to a synthetic medium containing the carbohydrate. He concluded, as did Bender and Levine (1942), Stein (1942), Stein et al. (1942), Bender (1942) and Liu (1952) that Pseudomonas fermentation can best be studied in a synthetic medium. Liu (1952) was of the opinion that the failure to produce an acid reaction in peptone-carbohydrate broth is due to the preference of the organism to utilize peptone as a source of carbon as well as the source of nitrogen. The results of Pseudomonas carbohydrate studies have been reported by many other workers including Jordan (1903), Seleen and Stark (1943), Gaby (1946) and Munoz et al. (1949).

Conn (1942) treated this topic of fermentation theoretically. He stated there is no question of fermentative ability when an organism produces acid in a peptone medium. However, if no acid is produced the organism may still be a fermenter. Actually it may be (1) unable to use the sugar, (2) using the sugar so completely that there are no products which give an acid

reaction to the medium except  $\text{CO}_2$ .

It was found very early in the study that many of the organisms in the collection would not grow well in any single synthetic medium devised. An attempt was made to devise a peptone-carbohydrate medium which would support good growth and still be capable of indicating acid end products. The organic medium devised has the advantage of comparing all the organisms on the same basis. The organisms respond well with growth whether the reaction under observation was positive or negative. Vera (1950) pointed out the care one must observe in choosing constituents for media since fermentable substances are contained in some peptones, yeast autolysates and beef extract. The effect of yeast extract, nitrates, phosphates, agar, peptone and dextrose concentration on the change of indicator in carbohydrate media was investigated. The following conclusions are based on this study with the nonfermenting group of organisms.

1. As the peptone concentration was increased from 0.5 to 5% the final acidity became increasingly less. Pancreatic digests of soy bean meal and gelatin were less satisfactory for incorporation in carbohydrate media. The soy peptone contained substances which the organisms were able to convert to acid end products. Oxidative acidity was in many instances completely masked when tryptic digest of gelatin was used.

2. Nitrates when reduced to nitrites or nitrogen increased the alkalinity of the medium.

3. Phosphates stabilized the pH.

4. Yeast extract had an effect similar to that of peptone. It contains substances which may be converted to acid end products by the

oxidizers.

5. The optimum concentration of dextrose and other carbohydrates is about 1%. A concentration of 0.5% dextrose or less was definitely inferior to 1% dextrose. A dextrose concentration of 3% or higher partially inhibited the growth of some of the organisms tested.

6. Agar at about 0.2% prevented convection currents and aided in intensifying and stabilizing the indicator color change.

Of the many combinations tried the formula below appeared most satisfactory.

pancreatic digest of casein (Bacto-Casitone)	0.5%
agar	0.2
carbohydrate (Seitz filtered)	1.0
final pH 7.1	

When those organisms which produced a surface acidity from dextrose are layered with petrolatum there is generally no growth or acid production. This is strikingly different from the behavior of members of the family Enterobacteriaceae. The Enterobacteriaceae grow well and produce a high acidity under the petrolatum. Based on this technique two types of carbohydrate metabolism may be distinguished namely, (1) aerobic (oxidation) and (2) anaerobic (fermentation). Restriction of the term "fermentation" to anaerobic metabolism of carbohydrates is in accordance with the original usage of Pasteur. While studying carbohydrate utilization by yeast, Pasteur discovered there were forms of life which do not require atmospheric oxygen, and stated, "La fermentation est la vie sans air". Fermentation then, in its original meaning, is a process whereby the organism, in the absence of air, obtains energy from carbohydrates.

Table IV compares growth and indicator changes of nonoxidizers, oxidizers and fermenters on the dextrose semisolid medium described. The medium was inoculated in duplicate with one of the tubes layered with petrolatum.

The nonfermenters are unable to grow in the dextrose-peptone medium when air is excluded with a petrolatum layer. However, the fermenters grow vigorously in a similar environment. These observations correlate with the indicator changes. The nonfermenters, being unable to grow, do not change the indicator; while the fermenters promptly change the indicator yellow even in the absence of air.

Nonfermenters can be recognized by indicator changes at the surface of the medium exposed to the air. They are divided into two groups, the (A) nonoxidizers and (B) oxidizers. The nonoxidizers produce an intense blue surface color and the oxidizers produce a yellow surface color change which after 4-7 days may extend to the bottom of the tube.

The fermenters quickly produce acidity throughout the entire tube in the presence of air, just as was observed under petrolatum. Facultative anaerobes can now be defined more definitively as organisms which can grow well with oxygen and can grow in the absence of oxygen provided fermentable carbohydrates are present which supply energy for growth. Whenever the term facultative anaerobe is used the compound or medium which allows the organism to grow in the absence of air should be stated. It appears that all the organisms in the family Enterobacteriaceae ferment dextrose under petrolatum and therefore are facultative.

Bacteria may then be separated into three units on the basis of aerobic or anaerobic metabolism of dextrose.



TABLE IV  
COMPARISON OF NONOXIDIZERS, OXIDIZERS AND FERMENTERS  
ON DEXTROSE MEDIUM

RH number	name	type of dextrose utilization	dextrose with petrolatum		dextrose	
			growth	color of indicator	growth	color of indicator
12	<u>Alcaligenes</u> sp.	nonoxidizers	-	nc	+	b surf
46	<u>Alcaligenes bronchicanis</u>		-	nc	+	b surf
125	<u>Pseudomonas</u> sp.		-	nc	+	b surf
2	<u>Pseudomonas</u> sp.	oxidizers	-	nc	+	y surf
176	<u>Agrobacterium radiobacter</u>		-	nc	+	y surf
218	<u>Pseudomonas</u> sp.		-	nc	+	y surf
410	<u>Bacterium anitratum</u> 5W2		-	nc	+	y surf
412	<u>Vibrio comma</u>	fermenters	+	y	+	y
253	<u>Aeromonas liquefaciens</u>		+	y	+	y
406	<u>Escherichia coli</u>		+	y	+	y
407	<u>Aerobacter aerogenes</u>		+	y	+	y
414	<u>Shigella paradysenteriae</u> IV		+	y	+	y
387	<u>Salmonella typhosa</u>		+	y	+	y

KEY:

b surf      deep blue surface, later becomes blue throughout the tube  
y surf      yellow surface (oxidation)  
y            yellow throughout the tube before 48 hours (fermentation)

## I. Nonfermenters

## A. Nonoxidizers

- a. some Pseudomonas
- b. Alcaligenes
- c. Alcaligenes bronchicanis
- d. parapertussis bacilli

## B. Oxidizers

- a. some Pseudomonas
- b. Xanthomonas
- c. Flavobacterium
- d. Agrobacterium

## II. Fermenters

- a. Salmonella
- b. Shigella
- c. Proteus
- d. Aerobacter
- e. Escherichia
- f. Vibrio comma
- g. Aeromonas

Organisms in the Pseudomonas-Alcaligenes group are generally unable to grow in the absence of air even if a utilisable carbohydrate is present in the medium, hence should be referred to as aerobic. Some of the organisms in this group can grow anaerobically if nitrates or similar compounds are present which can accept hydrogen from an oxidisable substrate. Some organisms in the group obtain energy anaerobically from amino acids and must be classified as facultative in this respect. These have not been studied in detail.

From Table IV it appears that the genus Aeromonas is physiologically unrelated to the genus Pseudomonas, and Bacterium anitratum is physiologically unrelated to the Enterobacteriaceae. It would be well to note too that Vibrio comma is capable of fermenting dextrose as do the Enterobacteriaceae hence should not be considered aerobic as is so often done. It grows

anaerobically in a peptone medium when dextrose is present. Physiologically Vibrio comma appears to be out of place in the family Pseudomonadaceae where the aerobic oxidizers as Pseudomonas, Xanthomonas, Acetobacter and Spirillum are classified. Vibrio comma shows a physiological and morphological relationship to the genus Aeromonas.

The reactions recorded in Table IV were controlled by duplicate inoculation in basal medium (lacking added carbohydrate). One of the duplicates was layered with petrolatum. The results are recorded in Table V.

The coliforms, typhoid and dysentery organisms produce moderate amounts of alkali from peptone that is similar in degree to that produced by Bacillus alcaligenes and Pseudomonas according to Kendall (1913) and Liu (1952). Table V shows that the fermenters produce no change of reaction in the open tubes. This probably means there were traces of fermentable substances in the peptone which were converted to acid end products, hence masking alkali production from peptone metabolism. In the presence of air these acid products were either further utilized to neutral end products or were neutralized by the increasing quantity of alkaline end products of peptone metabolism, possibly both occur simultaneously. This was further brought out by the reaction seen when the same medium was covered with petrolatum to exclude air. Here the acids produced were not neutralized or utilized, but the acidity was permanent. The growth in the closed tube was relatively poor as compared to that in the open tube. Probably the only energy available anaerobically for most Enterobacteriaceae is the fermentable substances in the peptone. The peptone under petrolatum did not serve as

TABLE V  
CARBOHYDRATE BASAL MEDIUM CONTROL

RH number	name	type of carbohydrate utilization	basal medium with petrolatum		basal medium	
			growth	color of indicator	growth	color of indicator
12	<u>Alcaligenes</u> sp.	nonfermenters	-	nc	+	b surf
46	<u>Alcaligenes bronchicanis</u>		-	nc	+	b surf
125	<u>Pseudomonas</u> sp.		-	nc	+	b surf
2	<u>Pseudomonas</u> sp.		-	nc	+	b surf
176	<u>Agrobacterium radiobacter</u>		-	nc	+	b surf
218	<u>Pseudomonas</u> sp.		-	nc	+	b surf
410	<u>Bacterium anitratum</u> 5W2		-	nc	+	b surf
412	<u>Vibrio comma</u>	fermenters	-	wk y	+	nc
253	<u>Aeromonas liquefaciens</u>		-	wk y	+	nc
406	<u>Escherichia coli</u>		-	wk y	+	nc
407	<u>Aerobacter aerogenes</u>		-	wk y	+	nc
414	<u>Shigella paradysenteriae</u> IV		-	wk y	+	nc
387	<u>Salmonella typhosa</u>		-	wk y	+	nc

KEY:

wk y      weak yellow

an energy source, since growth was less without air than with air. Most Enterobacteriaceae apparently are facultative only in the presence of a fermentable carbohydrate.

It may be noted here that this technique, of studying peptones under petrolatum with bromthymol blue indicator, is an excellent method of determining the presence of fermentable substances in peptones.

### MORPHOLOGY

Consideration here is limited to bacteria which are gram negative and rod shaped. Both of these characteristics may be indefinite and at times it appears impossible to classify an organism as a rod or a coccus, as gram negative or gram positive. No suggestions on this problem are presented and fortunately it is not very common. The size of the rod is not a very significant characteristic and is subject to considerable variation in different media and different growth phases. The shape of the rod is somewhat more important, particularly the curvature.

The pronounced curvature found in the spirochaetes and typical spirilla appears to be of taxonomic importance. Slight curvature must be treated cautiously. Some organisms show some curvature in certain media but no curvature in other media. A striking example is Vibrio comma which when cultivated in the laboratory often becomes perfectly straight. A slight curvature is also typical of the lophotrichous rods but the characteristic is not sufficiently pronounced to be more than of minor taxonomic importance. Considered together with flagellation, the minor curvature helps to indicate possible relationships.

If one examines several species of the commonly recognized spirilla one finds that the "cellular" units are often relatively short, slightly curved rods. A comparison of the morphology of Spirillum virginianum and the lophotrichous Alcaligenes shows a striking similarity in flagellation and body curvature. Lehmann and Neumann (1927) proposed the name Vibrio alkaligenes for the organism which now appears to be morphologically related to the spirilla. Pronounced pleomorphism, with tendency to form bizarre shapes and long filaments, does not appear to have much taxonomic importance in this group. The morphological characteristic of major importance appears to be flagellation. The various flagellar types were described by Leifson (1951) and have been demonstrated by photomicrographs in the presentation of the experimental data. The major significant categories are as follows:

1. peritrichous
2. polar multitrichous
3. lophotrichous
4. monotrichous
5. nonflagellated

Although the above arrangements are characteristic and stable properties of bacteria they are subject to changes. Leifson and Hugh (1953) described an organism (RH 247) which mutated from lophotrichous to peritrichous with intermediary forms having both types of flagellar arrangements. Another organism was also described which varied from monotrichous to peritrichous with intermediary types having both kinds of flagella. Just how common this phenomenon is cannot be stated at present but it is probably safe to say that it is somewhat unusual. It does emphasize however, the importance of not relying too much on any single characteristic in the classification of bacteria. The recognition of nonpolar flagella on predominantly polar

organisms was facilitated by the more "curly" shape of the lateral flagella as compared to the polar. It is possible that with some bacteria the flagella of the mutants may have the same shape, and would thus be more difficult to recognize. An odd flagellum which appears to come from the side of a polar type may be a polar flagellum which has been bent under the organism. The formation of multicellular filaments by a polar flagellate is another cause for confusion, Gray and Smith (1950), Leifson (1951 page 387 figures 23 and 24). The filaments appear to be peritrichously flagellated. The shorter units, which are usually present in any culture, establishes the flagellation.

The shape of bacterial flagella is generally stable and characteristic. The usual curvature is like that found on organisms such as Salmonella, Pseudomonas and Proteus. This shape appears remarkably constant and any great deviation immediately becomes apparent. The curvature of the flagellum however is not free from mutational change, Leifson (1951, page 381 figures 5 and 6). These studies indicate that the usual type of flagella for Salmonella can mutate to the less stable more tightly curved flagella.

A strain of physiologically similar monotrichous organisms (RH 339, 342 and 347) which had an unusually greater flagellar curvature was isolated from water. These flagella are strikingly different from the usual Pseudomonas types (see figure 5 under Experimental Data).

The flagella of the lophotrichous type usually has only one or two curves. This group is very distinctive in appearance and seems to form a natural group when considered together with the tendency of the bacterial body to curve.

The number of flagella, whether polar or nonpolar, appears by itself

to be of relatively minor taxonomic importance. More emphasis may be given to the number of flagella in the polar group than in the nonpolar. The lophotrichous types usually have two or more flagella. The polar multi-trichous organisms also produce two or more flagella. When the polar multi-trichous group is considered together with its physiological characteristics, the number of flagella appears to have some taxonomic value.

A consideration of the mutation from flagellated to nonflagellated types, and flagellated motile to flagellated nonmotile, Friewer and Leifson (1952), seems warranted. The later type was not found among the nonfermenters studied and it is not very important from the taxonomic standpoint provided the presence of flagella is not overlooked. Nonflagellated variants or mutants are fairly common and such mutation occurs frequently when handling cultures in the laboratory, particularly with the peritrichous types.

The organisms studied in this work which were found to have flagella were motile. Some of the organisms were nonmotile at 37°C but were motile when incubated at lower temperatures.

Tittsler and Sandholzer (1936) have proposed the use of a stab culture in semisolid agar for demonstration of motility. Motile organisms show a diffuse zone of growth spreading from the line of inoculation, non-motile cultures do not. This test is a good check on the hanging drop method but mainly applicable to organisms which are able to grow facultatively as the Enterobacteriaceae.

#### INDOL PRODUCTION

The production of indol from tryptophane is of considerable diagnostic value for the differentiation of fermentative types of bacteria.



but apparently not for the nonfermenters. All the nonfermenters tested gave a negative indol test.

#### METHYL RED

All the strains of nonfermenters tested gave a negative methyl red test.

#### VOGES-PROSKAUER

Approximately half the cultures in the collection were tested for acetylmethylcarbinol. All nonfermenters were negative.

#### CITRATE

The nonfermenters gave both positive and negative reactions on Simon's citrate agar. Typical pigmented Pseudomonas strains gave an unequivocal positive citrate test. However, among other groups the test was often difficult to interpret. This was especially true of the peritrichous and nonmotile nonoxidizers. The citrate reaction is of particular value in identifying nonpigmented or weakly pigmented Pseudomonas strains.

#### UREA

The hydrolysis of urea appears to be of considerable taxonomic value. Stuart, van Stratum, and Rustigian (1945) reported that Proteus in a strongly buffered medium could be differentiated from all other members of the enteric group by its action on urea. This is in agreement with Rustigian and Stuart (1941 and 1943) and Ferguson and Hook (1943).

Organisms received as Brucella abortus, Brucella suis, Brucella melitensis, Brucella (or Haemophilus) bronchiseptica (20 strains) and

parapertussis bacilli (3 strains) gave a positive urease test. Eldering and Kendrick (1952) reported that occasionally parapertussis bacilli were encountered which gave a negative urease test. The urease test might serve as an aid in the differentiation of the peritrichous respiratory tract organisms, bronchicanis, from other Alcaligenes species.

Excluding the organisms labeled Brucella and bronchiseptica the following 18 nonfermenters also gave a positive urease test in 48 hours.

	number of strains	RH numbers
peritrichous	5	29, 176, 225, 227, 283
lophotrichous	1	185
monotrichous	7	22, 259, 284, 308, 309, 310, 311
nonmotile	5	10, 15, 370, 373, 374

The value of the urease reaction for species differentiation of organisms concerned here, outside of the peritrichous nonoxidizers, is not yet clear. However, the reaction may also serve to separate a species among the nonmotile nonoxidizers.

#### GELATIN

Strains of bacteria sometimes are isolated which liquefy gelatin at first but lose this property upon continued cultivation. The rate and degree of liquefaction may be highly variable. With some strains the liquefaction is barely detectable and various techniques give conflicting results. This type of test is of limited value as a means of identifying bacteria.

Levine and Carpenter (1923) pointed out that the viscosity of autoclaved gelatin changes little for two or three days, but after this time it increases markedly for a week to ten days. A gelatinolytic organism may bring about liquefaction quite rapidly when inoculated into freshly prepared

gelatin but more slowly if inoculated into the same medium ten days or two weeks old. More of the gel in the latter instance must be transformed to the sol state to make liquefaction evident. These workers divided bacteria into three groups with respect to their action on gelatin:

1. Gelatin not hydrolyzed; no liquefaction.
2. Gelatin partially hydrolyzed and liquefied. Decomposition slow, if any.
3. Gelatin more completely hydrolyzed, and liquefied. Decomposition rapid, accompanied by marked increase in formal titration.

The four gelatin media, described in Materials and Methods, were compared simultaneously by the inoculation of selected organisms. Table VI shows that the detection of digestion of gelatin is a function of time and the medium employed. There is a wide range over which an organism can be considered positive or negative, since there is little agreement as to time of incubation or what media should be employed. Frazier (1926) suggested that gelatin stab liquefaction test has not been given a prominent place in classification because of its slowness and unreliability. However he suggested that this technique might be used if only those organisms that showed liquefaction after a week were considered positive.

TABLE VI  
GELATIN MEDIUM COMPARISON

days		2	5	7	14	21	28	49
RH number	medium number							
57	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	+	+
	3	-	-	-	-	+	+	+
	4	-	-					
122	1	-	-	-	+	+	+	+
	2	-	-	-	+	+	+	+
	3	-	-	+	+	+	+	+
	4	+	+					
10	1	-	-	-	-	-	-	-
	2	-	-	-	-	+	+	+
	3	-	-	-	-	+	+	+
	4	-	-					

#### HYDROGEN SULFIDE

Many indicators have been suggested for use in detecting hydrogen sulfide namely lead, iron, bismuth, nickel and cobalt, all of which produce a dark color of the sulfide of these metals. To overcome the toxic effect of heavy metals in media it has been suggested that a test strip of filter paper impregnated with a metallic salt be placed in the mouth of the tube during incubation.

It is generally agreed that the paper strip method is far more sensitive than many of the tests which incorporate the toxic indicator system in the media. The value of the paper strip method becomes limited since it gives so many positive reactions, for even such organisms as Shigella dysenteriae give a positive test. The test is influenced by the nature of

the peptone, and such substances as cystine and thiosulfate which may increase the number of positive reactions.

All the organisms studied were tested on Kligler's iron agar. The nonfermenters are generally unable to grow in the agar stab hence are unable to produce blackening in the butt. Some pseudomonads on prolonged incubation may show blackening in the line of growth on the slant. This is confirmed by Rozansky et al. (1950). Pulvirenti (1949) also observed  $H_2S$  production by B. pyocianum. However, it was found that many typical pigmented Pseudomonas strains do not give a positive  $H_2S$  test even with the paper strip technique. The hydrogen sulfide test seems of little taxonomic value for differentiation of the majority of nonfermenters.

Two unusual monotrichous organisms, RH 231 and 364, produced  $H_2S$  in the butt of Kligler's iron agar. Both of these nonfermenters probably obtain energy for anaerobic growth from amino acids.

#### NITRATE TO GAS

The reduction of nitrate has been widely used as a differential test. Nitrate may be decomposed beyond the nitrite stage and in many instances it is converted to molecular nitrogen. Other reduction products are apparently produced, but nitrites and nitrogen gas are most readily detected. The reduction of nitrates to nitrogen gas by bacteria is called denitrification. Microbial denitrification apparently is a process equivalent to aerobic oxidation with nitrate replacing oxygen as the ultimate oxidizing agent. A concentration of 0.2 to 0.5% nitrate in the medium is satisfactory for observing evolution of gas. The gas may be trapped in a Durham tube in

liquid medium. The formation of nitrogen bubbles in semisolid medium is not always reliable. Experiments conducted by Allen and van Niel (1952) with the denitrifying bacterium Pseudomonas stutzeri showed that neither amino acids, amides nor nitrogenous constituents of bacteria in the medium contribute to the nitrogen evolved during reduction of nitrate.

Nitrate reduction to gas was not frequently encountered among the nonoxidizers. The nonoxidizing denitrifiers were RH 12 and 13 (peritrichous) and RH 330 (monotrichous).

Among the dextrose oxidizers, nitrate reduction to gas is of taxonomic importance in the monotrichous group. Nitrogen gas production is particularly valuable in identifying pigmented pseudomonads as well as apyocyanogenic and afluorescigenic strains.

#### NITRATE TO NITRITE

The fifteen minute period of incubation as recommended by Brough (1950) for the determination of nitrate reduction does not seem adequate when studying nonfermenting organisms. An extension of the time to one hour was far more adequate since it gave more positive and fewer doubtful reactions.

#### ANAEROBIC GROWTH WITH NITRATE

Many organisms are capable of oxidizing organic substances in the absence of molecular oxygen by simultaneously reducing nitrate. Most compounds which serve as an oxidizable substrate under aerobic conditions are suitable energy sources in nitrate containing medium in the absence of air.

Nitrate in a 0.1% concentration supports excellent anaerobic growth

in peptone water for those aerobic organisms which convert nitrate to gas. The anaerobic growth of denitrifiers in the presence of nitrate was more rapid and profuse than that seen in media without nitrate exposed to the air. The anaerobic growth with nitrate is generally accompanied by copious gas production. This is well exemplified by organisms RH 12 and 13. With these organisms there is no visible anaerobic growth in peptone water in 18 hours but anaerobic growth with nitrate is heavier than that seen in nitrate free peptone exposed to the air.

Some of the organisms which convert nitrate only as far as nitrite show anaerobic growth in the presence of nitrate. This growth however is slower and less abundant than that described for true denitrifiers. This type of reaction is frequently seen among the lophotrichous organisms.

#### PIGMENTATION

Pigment production is of considerable diagnostic importance. The benefit derived from pigments by the organisms is not clear with the exception of those pigments which are essential to photosynthesis. The placing of excessive emphasis on pigmentation in taxonomy often raises a question of proper nomenclature for nonpigmented strains derived from pigmented organisms. Pigment production is not infrequently dependent upon the composition of the medium, temperature of incubation and oxygen. Among the faintly pigmented organisms color characterization is difficult. The factors essential for pigment production varies with the organism and no general medium can be devised which will be optimum in all cases. These factors indicate the disadvantages entailed in emphasizing pigmentation as an identifying character.

istic of some groups of organisms.

A study of the water soluble pigments of Pseudomonas has been made by Leifson (unpublished) and was found to be considerably enhanced in many cases by adding 0.01% manganese salts to a semisolid nutrient agar. The magnesium salts were less effective though also stimulatory. However, even with the manganese medium a number of strains having all the other characteristics of Pseudomonas aeruginosa still failed to produce a water soluble pigment. Several papers have recently been published on the Pseudomonas pigments, Selezn and Stark (1943), Burton et al. (1947 and 1948), King et al. (1948), Rozansky et al. (1950), Harris (1950), Haynes (1951), Hellinger (1951), Avezzu (1951), and Totter and Moseley (1953).

Rozansky et al. (1950), who apparently were working with highly pigmented strains of pseudomonads, observed pigment to be abundantly produced on Loeffler's medium. Many times Loeffler's medium showed no pigment, when obviously pigmented strains were employed. In three instances RH 187, 188 and 189 pigmentation was observed on Loeffler's medium but not on any other medium tried. Loeffler's medium has an inherent disadvantage since it is often rapidly liquefied before pigment develops on the slant.

#### MILK AGAR CLEARING

Clearing of milk agar is perhaps more sensitive and reliable than litmus milk as an indication of proteolysis. There is no assurance in this test that casein is cleared only by proteolysis. Clearing of the agar was observed after 24 and 48 hours.



BLOOD AGAR

Henriksen (1937) drew attention to hemolytic strains of nonmotile Alcaligenes. The Alcaligenes hemolysans described produced a soluble, antigenic hemolysin which was inactivated at 50°C in  $\frac{1}{2}$  hour. Several strains of nonmotile organisms called Alcaligenes hemolysans were received from Henriksen and Oeding in Norway (RH 10, 164, 165, 166, 167, 168, 169 and 170). These eight strains produced a beta hemolysis about subsurface colonies, as well as hemolytic zones about the surface colonies. RH 10 and 168 oxidized carbohydrates, while the others were nonoxidizers. In addition to the above strains five other organisms with the same type of hemolysis were encountered in the study. The various beta hemolytic strains are listed in Table VII with some of their characteristics.

Deep colonies of all the remaining organisms studied were non-hemolytic with the following exceptions. The peritrichous organisms RH 135 and 223 produced an alpha hemolysis. The monotrichous organisms RH 188 and 231 produced an alpha hemolysis. Alpha hemolysis was also observed among the nonmotile organisms.

TABLE VII

## BETA HEMOLYTIC NONFERMENTING GRAM NEGATIVE RODS

RH number	received as	source	flagellation	citrate	gelatin	nitrate	dextrose
10	<u>Alcaligenes hemolysans</u>	spinal fluid	-	+	-	-	+
164	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
165	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
166	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
167	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
168	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	+
169	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
170	<u>Alcaligenes hemolysans</u>	throat	-	+	+	-	-
186		stool	-	-	-	-	-
194		stool	-	+	-	-	-
197		stool	polar multi	+	-	-	+
210		blood	mono	+	+	●	+
216	<u>Alkaligenes faecalis</u>	stool	-	+	+	-	-

LITMUS MILK

Milk has been used for the study of microorganisms since the beginning of bacteriology. The usefulness of milk was increased by the addition of a suitable indicator such as litmus. Litmus has an advantage over many indicators for it is reduced by some bacteria. It is, however, not too sensitive to slight changes of reaction.

As early as 1889 Petruschky recognized the limitations of differentiating bacteria on the basis of milk reactions. He used a litmus whey medium which was free from casein for the differentiation of Bacillus faecalis alcaligenes and the typhoid bacillus.

Ayers and Johnson (1913) and Ford (1903) observed that in litmus milk often enough alkali was produced by organisms to dissolve the casein. This happened on prolonged periods of incubation. Ayers et al. (1919) believed the alkaline reaction to be due almost entirely to formation of alkaline carbonates from the oxidation of the salts of organic acids, presumably citric acid. This is in agreement with the earlier work of Seiffert and Wymer (1912).

At least 5 or 6 reactions may occur in litmus milk. They are dextrose, lactose and citrate metabolism, reduction of litmus, caseolysis, rennet and acid coagulation of casein. Simpler media have been devised for the study of carbohydrate metabolism without confusing this reaction with other activities which also may be taking place in the same tube simultaneously.

The Alcaligenes bronchicanis strains and other nonfermenters produce an intense alkaline reaction sufficient to dissolve the casein. The

appearance of the medium was not unlike that seen with proteolytic organisms.

The litmus milk reaction does not seem to add to the identifying characteristics of the organisms when it is understood that the alkali production arises from citrate utilization or nitrogen metabolism. Some of these reactions were studied individually with a more suitable medium.

#### DESOXYCHOLATE

The photomicrographs published by Kuhnemann (1911) shows that he was able to isolate only lophotrichous organisms from fecal material. Leifson (1935) stated he was unable to isolate, from fecal material streaked on bile agar, the peritrichous Alcaligenes but lophotrichous organisms were encountered. These reports seem to indicate that it is the lophotrichous "Alcaligenes" which is encountered in fecal studies using bile media.

Growth on 0.1% desoxycholate agar was studied to formulate an idea of the natural habitat of nonfermenting organisms, assuming failure to grow as an indication of nonintestinal origin. The following generalizations could be made:

1. The lophotrichous "Alcaligenes" grew well.
2. The bronchicanis strains grew very poorly, if at all; however, 6 peritrichous nonoxidizers (RH 12, 13, 222, 223 and 232) grew well.
3. Pigmented pseudomonads grew well. Some of the nonoxidizing monotrichous organisms did not grow.

#### GROUPING OF THE ORGANISMS

The organisms concerned in this treatise, for systematic consideration, were divided into three units on the basis of ability to use dextrose:

Unit A nonoxidizers (120 organisms)  
Unit B oxidizers (123 organisms)  
Unit C fermenters (39 organisms)

Unit A was further divided into five groups.

Group 1 peritrichous  
Group 2 polar multitrichous  
Group 3 lophotrichous  
Group 4 monotrichous  
Group 5 nonmotile

Table III shows the separation of the organisms listed in Table II into wields units, subunits and groups, which will be considered individually.

Group 1 of Unit A was comprised chiefly of organisms received labeled Brucella bronchiseptica or Haemophilus bronchisepticus (the first 20 organisms listed in Table VIII). It was observed that without exception all 20 strains labeled in this way were morphologically similar. All gave a positive urease test. Physiologically they differed in their action on nitrate. The characteristics of the 20 strains was in agreement with the delineation of Brucella bronchiseptica as worked out by Eldering and Kendrick (1952).

The detailed characteristics of the peritrichous nonoxidizers are shown in Table VIII. In addition to the 20 bronchicanis strains, 3 other organisms were encountered which appear to belong to the bronchicanis group (the first 23 organisms listed in Table VIII). It might be well to draw special attention to the organism received labeled Bacterium alcaligenes, RH 225, isolated from the lungs of a guinea pig. This organism has all the morphological and physiological characteristics of the bronchicanis strains, but the citrate reaction is negative. Should such an organism be thrown out of the bronchicanis group because of the citrate reaction? Organisms RH 29

TABLE VIII  
PERITRICHOUS NONOXIDIZERS

RH numbers	dextrose lactose sucrose maltose mannose xylose arabinose indol methyl red Voges-Proskauer urea nitrate citrate gelatin H <sub>2</sub> S H <sub>2</sub> O sol. pigment milk agar
48, 49, 50, 51, 183, 184, 214	- - - - - - - - - - + - + - - - -
46, 47, 52, 53, 54, 140, 171, 172, 173, 180, 181, 182, 232	- - - - - - - - - - + + + - - - -
29, 225, 227	- - - - - - - - - - + + - - - - -
338, 336	- - - - - - - - - - - - - + - - +
322 yellow water insoluble pigment	- - - - - - - - - - - - - + - - +
222	- - - - - - - - - - - - + + - - -
135, 223, 258, 376, 378	- - - - - - - - - - - - + - - - -
138, 260	- - - - - - - - - - - - + - - - -
12, 13	- - - - - - - - - - - - 0 + - - - -
379, 380	- - - - - + - - - - - - 0 + - - - -
60, 83, 136, 137	nc - - - - + - - - - - - 0 + - - - -

TABLE IX  
THE RELATIONSHIP OF NITRATE AND SOURCE OF ISOLATION  
AMONG 23 ALCALIGENES BRONCHICANIS STRAINS

RH number	source	nitrate	RH number	source	nitrate
29		+	172	guinea pig	+
46	dog	+	173	guinea pig	+
47	dog	+	180		+
48	pig	-	181	human	+
49	pig	-	182	murine	+
50	pig	-	183	canine	-
51	pig	-	184		-
52	dog	+	214	dog	-
53	dog	+	225	guinea pig	+
54	dog	+	227	human blood	+
140		+	232	dog	+
171	guinea pig	+			

and 227 though of different origin are very similar to RH 225.

Simmons and Koser citrate reactions, for the peritrichous non-oxidizers, in many instances were not a definite positive or negative. These citrate reactions leave much room for interpretation, hence probably are not a sound basis for species differentiation.

The relationship of nitrate reduction to the source of isolation of these organisms is shown in Table IX. Generally strains isolated from the dog gave a positive nitrite test while those from the pig were negative.

The variation in the citrate and nitrate reactions should probably be considered as variations within a species. The nitrate reaction has been recognized by Torrey and Rahe (1912-1913) as being variable among the bronchicanis strains, hence this variation within the species is not a new departure in systematics.

There is little justification for the separation of genera on the basis of source of isolation. All nonoxidizing peritrichously flagellated organisms may be classified as Alcaligenes. It appears that the reason for not considering bronchicanis as belonging to the genus Alcaligenes was the undue significance given to the source of isolation. During the time when bronchicanis was first being described it did not seem logical to include organisms isolated from the respiratory tract in the same genus as Alcaligenes, which was erroneously thought to have an exclusive fecal habitat. Furthermore many bacteriologists at that time considered Bacillus faecalis alcaligenes to be polar flagellated, Berghaus (1905), Kleminko (1907), Kuhnemann (1911), Glasser and Hachla (1911), Horn and Huber (1911), and Baerthlein (1912).

The sources of isolation of the peritrichous nonoxidizing organisms

in Unit A Group 1, exclusive of those urea positive organisms considered to be Alcaligenes bronchicanis, are given in Table X.

TABLE X  
SOURCE OF ISOLATION OF PERITRICHOUS NONOXIDIZERS

RH numbers	source	received as
12 and 13	soil	<u>Alcaligenes</u> sp.
135 and 378		<u>Alcaligenes faecalis</u>
138	ropy milk	<u>Alcaligenes viscosus</u>
222	feces	<u>Bacterium alcaligenes</u>
223		<u>Bacterium alcaligenes</u>
258	feces	<u>Alcaligenes</u> sp.
260	variant of 247	<u>Vibrio percolans</u>
322*	stream	
336 and 338	stream	
376		<u>Alcaligenes</u> sp.

\* produces a water insoluble yellow pigment.

The author recommends that the organisms in Table X, since they have the same morphology and similar physiology, should be placed in the same genus together with bronchicanis irrespective of the source of isolation. These organisms with different sources of isolation, whether it be soil, respiratory tract or feces, should not be placed in different genera unless there exists taxonomically significant differences. Such taxonomic differences have not been demonstrated.

The genus Flavobacterium has been created for water insoluble yellow peritrichous nonfermenters. Organism RH 322, shown in Table VIII, by the above definition belongs to the genus Flavobacterium. Pigmentation as considered earlier is often an unstable characteristic and nonpigmented variants are readily isolated from pigmented strains. Aside from pigmentation



the characteristics of RH 322, both anatomical and physiological, correspond to the Alcaligenes faecalis strains 336 and 338.

Organism RH 260 was particularly interesting since Leifson and Hugh (1953) reported it's isolation from RH 261 which had lophotrichous flagella. The lophotrichous organism RH 261 was isolated from organism RH 247 which was described as Vibrio percolans by Mudd and Warren (1923).

The second group of 11 organisms in Table VIII can become known as Alcaligenes faecalis (RH 338, 336, 322, 222, 135, 223, 258, 376, 378, 138 and 260). Alcaligenes faecalis can be described to have the following characteristics: Gram negative, peritrichously flagellated, asporogenous aerobic rods. Does not oxidize or ferment carbohydrates. Indol, methyl red, Voges-Proskauer and urea tests negative. Nitrates may be reduced to nitrites but not to gas. The citrate and gelatin reactions are variable. The hydrogen sulfide test is negative on Kligler's iron agar. The author is not averse to including yellow water insoluble pigmented organisms as RH 322, with the above characteristics in the species faecalis.

Two organisms isolated from soil, RH 12 and 13, could readily be distinguished from all other peritrichous nonoxidizers since they reduced nitrates to nitrogen gas. The name Alcaligenes denitrificans sp. nov. is proposed for those true denitrifiers.

The six organisms, RH 60, 83, 136, 137, 379, 380, listed at the bottom of Table VIII are considered separately from the other peritrichous nonoxidizers. These organisms were unusual in that they produced oxidative acidity from xylose. It may not be out of place to speculate that all 6 strains were of the same origin, since the oldest culture, RH 137, was de-

posited in the American Type Culture Collection in 1925 and the origin of the remaining strains is not certain. The physiology of the organisms RH 60, 83, 136 and 137 appears different than the majority of the dextrose nonoxidizers. Interpretation of the dextrose reaction by the technique employed was difficult for the organisms did not produce the usual abundance of alkali characteristic of nonoxidizers but left the reaction of the medium unchanged or only very faintly acid.

The validity of the meager description of Bacillus faecalis alcaligenes left by Petruschky can be doubted for the following reasons:

- (1) Petruschky recorded only gas production from carbohydrate media studies.
- (2) Proof by Petruschky's contemporaries that strains of Petruschky's Bacillus faecalis alcaligenes were contaminated with typhoid bacilli, Pseudomonas and other water insoluble pigmented organisms. Should one accept Petruschky's characteristics of Bacillus faecalis alcaligenes without question of validity, one can justifiably doubt that the characteristics recorded by Petruschky were complete enough to reidentify his organism. This is in agreement with Conn (1942) since he too was not certain that this organism could be recognized from the description given by Petruschky.

After careful consideration of the literature it appears that the characteristics of Bacillus faecalis alcaligenes as given by Petruschky (1889 and 1896) also fit the description of the homogeneous group of organisms being called Brucella bronchiseptica. Torrey and Rahe (1912-1913) recognized that the litmus milk and potato reactions were similar for both bronchiseptica and Bacillus faecalis alkaligenes.

Wilson and Miles (1946), and Smith and Conant (1952), place the

bronchiseptica organism in the genus Haemophilus because of common antigens as found by Eldering and Kendrick (1937 and 1938), Huddleson (1939), Floedorf et al. (1941), Parfentjev et al. (1947); common toxins as found by Evans (1940); and the type of respiratory disease produced in animals and man as found by Brown (1926). Pathogenicity and antigenicity have a greater taxonomic significance than morphology and physiology when bronchiseptica is placed in the genus Haemophilus.

Manometric studies, by McCullough and Beal (1951) and Altenbern and Housewright (1952) have confirmed earlier reports by McAlpine and Slanetz (1928) and McCullough and Dick (1943) on carbohydrate utilization by strains of Brucella abortus, Brucella suis and Brucella melitensis. Using the medium and technique defined above fifteen strains (RH 42, 43, 44, 45, 67, 68, 69, 70, 71, 114, 115, 117, 118, 119 and 120) of abortus, suis and melitensis were found to produce oxidative acidity from carbohydrates. There exists then a marked difference in the physiology of these three Brucella species and the bronchiseptica strains, which do not metabolize carbohydrates. This physiological difference indicates that bronchiseptica should no longer be retained in the genus Brucella in spite of the similarity of the nucleoprotein.

Eldering and Kendrick (1952) realizing the antigenic relationship of the parapertussis bacillus to both Haemophilus pertussis and Brucella bronchiseptica, emphasized that these three organisms are misfits in the genus Haemophilus and suggest that they be placed together in a different genus. Anderson (1952) confirmed the serological relationship among these three organisms by cross-absorption tests using O and K antigens and cross-neutralization tests with toxin-antitoxin. These organisms were identical with

regard to the thermostable common O antigen, and haemorrhagic toxin, whereas each of them possesses its own specific K antigen and additional overlapping K antigens.

It appears justifiable to recommend that bronchiseptica be placed, as in Bergey's Manual of Determinative Bacteriology, 4th edition (1934), in the genus Alcaligenes and become known as bronchicanis, since this name has priority. Consideration should be given to making bronchicanis Ferry (1910, 1911 and 1912) and M'Gowan (1911), a very precisely described organism, the type species of the genus Alcaligenes. There are no discrepancies with the description of the probably unidentifiable Bacillus faecalis alcaligenes, Petruschky and the description of bronchicanis.

#### UNIT A GROUP 2

This polar multitrichous group, Table XI, shows a close relationship to both Pseudomonas and Xanthomonas. The existing description of these two genera are quite vague to be certain which genus should be assigned. A species distinction in one of these genera is warranted for the 6 strains not utilizing dextrose. These organisms did not produce a water soluble pigment but four produced a weak or strong water insoluble yellow pigment while two produced no pigment. The resemblance to Xanthomonas suggests itself because of the yellow pigment, however Xanthomonas is described as being predominantly monotrichous.

It is recommended that the polar multitrichous dextrose nonoxidizers, RH 294, 287, 430, 431, 229 and 205, become known as Xanthomonas maltophilia sp. nov. They can be described as having the following characteristics:

TABLE XI

## POLAR MULTITRICHOUS DEXTROSE NONOXIDIZERS

RH numbers	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	nitrate	H <sub>2</sub> O sol. pigment	milk agar
294	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
287*	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
430, 431*	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	-
229	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	+
205*	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-
162*	nc	-	-	+	+	-	-	-	-	-	-	-	+	-	-	-	+
7	nc	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+

\*produces a yellow water insoluble pigment

Gram negative, asporogenous, aerobic rods with polar multitrichous flagellation.

Do not oxidize or ferment dextrose, lactose, sucrose, xylose or arabinose.

Oxidative acidity is produced from maltose. Mannose may or may not be oxidized. Indol, methyl red, Voges-Proskauer, citrate and urea tests are negative.

Nitrates may be reduced to nitrites but not to gas. Gelatin is generally

liquefied. The hydrogen sulfide test is negative on Kligler's iron agar. A

yellow water insoluble pigment may be produced. These organisms were isolated

from stream and stool cultures. One strain may have been isolated from pleural

fluid.

Among the polar multitrichous organisms listed in Table XI are RH 7 and 162. These unusual organisms gave no change of indicator or possibly a faint trace of acid in the dextrose medium. When either sodium chloride or phosphate were incorporated in the same medium organism RH 162 unmistakably oxidized dextrose. Organism RH 7 could not be similarly encouraged.

### UNIT A GROUP 3

The lophotrichous nonoxidizers are listed in Table XII. Two strains, RH 274 and 439, of the photosynthetic organism Rhodospirillum rubrum were included in the table for comparative purposes. The remaining strains in the table are fairly homogeneous. This group has long been considered as Alcaligenes faecalis. Leifson (1942 and 1951) was in agreement with Kuhnemann (1910 and 1911) for both published excellent photomicrographs of similar organisms of fecal origin. These organisms were in general less sensitive to bile salts than the peritrichous Alcaligenes. They were labeled Vibrio alcaligenes by Lehmann and Neumann (1927) after the work of Strecker (1917). Nyberg (1935), his Group B, and Severi (1949) also encountered Vibrio alcaligenes. The genus designation Vibrio is unsuited for these organisms since they do not ferment dextrose.

Spirillum virginianum, RH 273, is included here among the lophotrichous nonoxidizers since there is similar flagellar and somatic morphology as well as similar physiology.

TABLE XII  
LOPHOTRICHOUS NONOXIDIZERS

RH numbers	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	citrate	urea	gelatin	H <sub>2</sub> S	nitrate	H <sub>2</sub> O sol. pigment	milk agar
274, 439	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
273	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
17, 31, 32, 37, 124, 131, 133, 212, 226, 261, 385	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
185	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
247*	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

\*PERITRICHOUS AND LOPHOTRICHOUS see RH 260 and 261

Gray and Thornton (1928) described Pseudomonas dacunhae n. sp., which did not produce acid from carbohydrates (see History, Gray and Thornton 1928). The figures they drew describing the morphology show curved rods with lophotrichous flagella. Contrary to Bergey's Manual of Determinative Bacteriology, 6th edition (1948 page 105) the gram stain was not recorded and nitrates were not reduced to nitrites.

Tataroff (1891) described Bacillus aquatilis sulcatus (Weichselbaum) as a motile sporulating rod 3.5 to 5 microns long, producing a water soluble

blue-green pigment. Migula (1900 page 933 and Table XIII figure 5) printed a photomicrograph showing the lophotrichous flagellation of Pseudomonas aquatilis. Bergey's Manual of Determinative Bacteriology, 6th edition (1948 page 146) carries Pseudomonas aquatilis in Appendix I of the genus Pseudomonas. The author has never encountered a lophotrichous water soluble pigment producer.

Although, according to the literature, the lophotrichous strains are predominantly of fecal origin there seems to be some indication that they may be ubiquitous. Table XIII shows the source of isolation of the strains studied.

TABLE XIII

## SOURCE OF ISOLATION OF LOPHOBACTER FAECALIS

RH numbers	source of isolation	received as
17, 31, 124	stool	<u>Alcaligenes</u> sp.
32		<u>Alcaligenes</u> sp.
37	water	<u>Alcaligenes</u> sp.
131, 133	stool	
212	blood	
226		<u>Bacterium alcaligenes</u>
273		<u>Spirillum virginianum</u>
261	variant of 247	<u>Vibrio percolans</u>
385	abdominal swab	<u>Alcaligenes</u> sp.

The name Lophobacter faecalis gen. et sp. nov. is suggested for the



following twelve strains, RH 273, 17, 31, 32, 37, 124, 131, 133, 212, 226, 261 and 385, listed in Tables XII and XIII. It is described as follows: Gram negative, asporogenous, aerobic rods with lophotrichous flagellation. Does not oxidize or ferment dextrose, lactose, sucrose, maltose, mannose, xylose or arabinose. Indol, methyl red, Voges-Proskauer, citrate and urea tests negative. Nitrates are generally reduced to nitrites but not to gas. Gelatin may be liquefied. The hydrogen sulfide test is negative on Kligler's iron agar.

#### UNIT A GROUP 4

Frequently nonoxidizing monotrichous organisms are considered to be members of the genus Alcaligenes. Table XIV shows the reactions of the monotrichous dextrose nonoxidizers. A photosynthetic Rhodopseudomonas, RH 275, was included in the table for comparative purposes. None of these organisms, with the exception of RH 25 and 26 produced a water soluble pigment. Monias (1928) suggested that monotrichous organisms which did not produce acid from carbohydrates should be called Pseudomonas alcaligenes. However, it did not appear that he differentiated the lophotrichous Vibrio alcaligenes of Lehmann and Neumann. The name Pseudomonas alcaligenes seems well suited for organisms RH 6, 21, 22, 27, 28, 125, 126, 330, 25 and 26.

In addition to the above nonoxidizing monotrichous pseudomonads three strains were encountered, RH 339, 342 and 347, which were like the above in most respects save for the shape of the monotrichous flagellum. The single polar flagellum was strikingly different than that seen on the usual Pseudomonas for it was "curly". The wave length of the flagellum was

TABLE XIV

## MONOTRICHOUS DEXTROSE NONOXIDIZERS\*

RH numbers	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	methyl red	Voges-Proskauer	citrate	urea	gelatin	H <sub>2</sub> S	nitrate	H <sub>2</sub> O sol. pigment	milk agar
275 <sub>a</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-
22	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-
27, 28, 125, 126	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
330	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-
25, 26 <sub>b</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
339, 342, 347 <sub>c</sub>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
38 <sub>d</sub>	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+
231 <sub>e</sub>	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-
249	-	-	+	-	+	-	-	-	-	-	-	-	0	-	-	-
268 <sub>f</sub>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
269 <sub>fg</sub>	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+

- \* The indol test was negative for all the organisms listed.  
a Rhodopseudomonas  
b brown water soluble pigment  
c curly flagella  
d rose-black water insoluble pigment  
e pink-orange water insoluble pigment  
f monotrichous and peritrichous  
g yellow-orange water insoluble pigment

considerably shorter in comparison to the more usual type. The shape variation can be seen by comparing the photomicrographs, Figures 4 and 5, on page 107. Perhaps this phenomenon might be related to the flagellar variant of Salmonella wicheta photographed by Leifson (1951 figure 6 page 380). A species distinction in the genus Pseudomonas seems justified.

Three other monotrichous organisms were encountered which may be more closely related to the dextrose oxidizers. RH 38 oxidized maltose and produced a water insoluble rose-black pigment. RH 231 oxidized arabinose but likewise did not oxidize dextrose. Pseudomonas saccharophilia, RH 249, which oxidized sucrose and mannose, is better known by the work of Doudoroff (1940).

Organisms RH 268 and 269 were nonoxidizing organisms difficult to place in any genus because of their flagellar morphology. They were predominantly monotrichous organisms with scattered peritrichous cells showing more convoluted lateral flagella. They have been photographed by Leifson and Hugh (1953). Pure peritrichous or monotrichous strains could not be maintained in culture.

Bergey's Manual of Determinative Bacteriology, 6th edition (1948) describes several species of Pseudomonas which appear to have some relationship to the monotrichous nonoxidizers in Table XIV. Some of them are:

<u>Pseudomonas cruciviae</u>	page 103
<u>Pseudomonas stisolobii</u>	page 135
<u>Pseudomonas astragali</u>	page 139
<u>Pseudomonas maublancii</u>	page 140
<u>Pseudomonas iridicola</u>	page 140
<u>Pseudomonas panici-miliacei</u>	page 143
<u>Pseudomonas erlobotryae</u>	page 144

Although Bergey's Manual of Determinative Bacteriology, 6th edition

(1948) describes the monotrichous genus Xanthomonas as producing acid from monosaccharides and disaccharides, the species alfalfae (page 165), phormicola (page 166) and nigromaculans (page 168) are described as not utilizing carbohydrates. These too then may be related to the organisms listed in Table XIV.

#### UNIT A GROUP 5

The last group of organisms to be considered in Unit A are the nonmotile dextrose nonoxidizers. One can recognize the limitations of assigning nonmotile nonoxidizing organisms to one of the four flagellated dextrose nonoxidizing groups for they may have evolved from any of these groups. Perhaps a serological study of somatic antigens could offer some help in assigning these nonmotile organisms to the proper flagellated group. Table XV shows the characteristics of the nonmotile dextrose nonoxidizers. Table XVI shows their source of isolation.

Four authentic strains of nonmotile Alcaligenes viscosus, RH 15, 23, 35 and 36, were studied and listed in Table XV. Organisms isolated from feces, blood and water were very similar to these strains of Alcaligenes viscosus.

Nine nonoxidizing beta hemolytic strains of Alcaligenes were studied. Six of these strains, RH 164, 165, 166, 167, 169 and 170, isolated from the throat, were received as Alcaligenes hemolysans.

The author has chosen to recognize the first 25 organisms of Table XV as Alcaligenes viscosus. Considerable significance is placed on the finding of the beta hemolytic fecal organism, RH 194, which appears to be similar

TABLE XV  
NONMOTILE DEXTROSE NONOXIDIZERS \*

RH number	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	citrate	urea	gelatin	milk agar	blood agar deep	H <sub>2</sub> O sol. pigment
14, 18, 63, 64	-	-	-	-	-	-	-	-	-	-	-	-	-
186	-	-	-	-	-	-	-	-	-	-	-	B	-
245	-	-	-	-	-	-	-	-	-	-	-	-	-
19, 23, 35, 36, 127, 211	-	-	-	-	-	-	-	+	-	-	-	-	-
9, 110, 128, 132, 160	-	-	-	-	-	-	-	+	-	-	-	A	-
194	-	-	-	-	-	-	-	+	-	-	-	B	-
216	-	-	-	-	-	-	-	+	-	+	-	B	-
164, 165, 166, 167, 169, 170	-	-	-	-	-	-	-	+	-	+	+	B	-
370, 373, 374	-	-	-	-	-	-	-	-	+	-	-	-	+ brown
15	-	-	-	-	-	-	-	+	+	-	-	-	-
201	-	-	-	-	-	-	-	+	-	-	-	A	+ brown
107	nc	-	+	-	-	-	-	-	-	-	-	-	-
62, 65, 139, 175	nc	nc	nc	nc	nc	nc	nc	-	-	-	-	-	-

\* Indol, methyl red, Voges-Proskauer, hydrogen sulfide and nitrate tests were negative.

to the authentic Alcaligenes viscosus and Alcaligenes hemolysans strains studied. The entire group warrants further investigation before hemolytic strains are separated from Alcaligenes viscosus as a new species. Alcaligenes viscosus can be described as having the following characteristics: Gram negative, asporogenous, aerobic, nonmotile rods without flagella. Does not oxidize or ferment dextrose, lactose, sucrose, maltose, mannose, xylose, or arabinose. Indol, methyl red, Voges-Proskauer, urea, hydrogen sulfide and nitrate tests negative. Citrate and gelatin tests variable. Blood agar deep colonies may show an alpha, beta or gamma hemolysis.

TABLE XVI

## SOURCE OF ISOLATION OF NONMOTILE NONOXIDIZERS

RH numbers	source	received as
9, 127		
14, 139, 175, 245		<u>Alcaligenes faecalis</u>
15, 23, 35, 36		<u>Alcaligenes viscosus</u>
18, 19	frog tank water	
62, 63, 64, 65	feces	<u>Heliconema</u> sp.
107, 128	water	
110, 216	feces	<u>Alcaligenes faecalis</u>
132, 211	blood	
160, 186, 194, 201	feces	
164, 165, 166, 167, 169, 170	throat	<u>Alcaligenes hemolysans</u>
370, 373, 374		parapertussis bacillus

The Alcaligenes viscosus strains studied by Long and Hammer (1935) and those listed in Table XV gave negative nitrate tests. Abd-el-malek and Gibson (1952) found nitrate positive strains. The majority of cultures examined by Abd-el-malek and Gibson did not produce aropy condition in milk. Viscosity is described as an indefinite property varying in degree from strain to strain and among colonies isolated from a single culture. The oxidation of citrate, and hence the production of alkalinity in litmus milk, was also shown to be slow and erratic.

Four strains, RH 201, 370, 373 and 374 produce a brown water soluble pigment. The latter three strains are parapertussis bacilli while RH 201 is of fecal origin. RH 15 and 201 are similar to the parapertussis bacillus. These strains may become known as Alcaligenes parapertussis. Eldering and Kendrick (1952) recorded a positive citrate reaction for parapertussis bacilli. The strains RH 370, 373 and 374 gave a negative Simmon's citrate reaction.

Organisms RH 62, 65, 107, 139 and 175 produced no change of indicator or only a very faint trace of acid in the dextrose medium. This was quite unusual since the majority of organisms produced an acid or alkaline reaction in this medium.

The paramount objective of this dissertation was not the ultimate organization of the carbohydrate oxidizers. However, generally unrecognized significant differences were encountered which cannot be minimized when organization in this group is to be considered. The oxidizers were investigated for (1) orientation, (2) confidence as to what should or should not be called Alcaligenes, (3) what organisms are concerned when Alcaligenes and

related generic names have been applied. Unit B was comprised of 123 organisms which oxidized dextrose and was divided into IV Subunits on the basis of oxidation of lactose and sucrose. Table XVII shows how Unit B was divided into the IV Subunits. The subunits were further separated into groups on the basis of flagella as was done in Unit A.

TABLE XVII DIFFERENTIATION OF DEXTROSE OXIDIZERS (UNIT B) INTO SUBUNITS			
subunit	dextrose	lactose	sucrose
I	+	-	-
II	+	-	+
III	+	+	-
IV	+	+	+

#### UNIT B SUBUNIT I GROUP 1

Only one peritrichous organism was found in Group 1. This was the organism Acetobacter aceti, RH 262, apparently a misnomer for the entire genus is considered by Vaughn (1943) to be monotrichous if motile. Photomicrographs showing the flagellation of Acetobacter are generally lacking, those published by Vaughn (1942) and Zeidler (1898) are unconvincing.

According to Vaughn (1942 and 1943) there exists a very close relationship between Acetobacter and Pseudomonas. The two genera probably differ in their utilitarian significance. Acetic acid bacteria can grow at an extremely low pH and can oxidize 6% alcohol to acetic acid.

Two Acetobacter strains, RH 263 and 277, were compared simultan-



TABLE XVIII  
OXIDATION OF ETHANOL

RH number	received as	flagellation	dextrose	ethanol		
				1%	3%	5%
1	<u>Pseudomonas aeruginosa</u>	mono	+	+ <sub>2</sub>	+ <sub>2</sub>	+ <sub>2</sub>
3	<u>Pseudomonas aeruginosa</u>	mono	+	+ <sub>2</sub>	+ <sub>2</sub>	+ <sub>2</sub>
5	<u>Alcaligenes faecalis</u>	polar multi	+	+	+	+
9		-	-	+	+ <sub>3</sub>	+ <sub>3</sub>
21	<u>Alcaligenes bookeri</u>	mono	-	+	+ <sub>2</sub>	+ <sub>2</sub>
28	<u>Alcaligenes sp.</u>	mono	-	+ <sub>2</sub>	+ <sub>2</sub>	+ <sub>2</sub>
35	<u>Alcaligenes viscosus</u>	-	-	+	+	+ <sub>2</sub>
66	<u>Alcaligenes sp.</u>	mono	+	+ <sub>4</sub>	+ <sub>4</sub>	+ <sub>4</sub>
122	<u>Pseudomonas aeruginosa</u>	mono	+	+ <sub>4</sub>	+ <sub>4</sub>	+ <sub>4</sub>
127		-	-	-	-	-
177	<u>Agrobacterium tumefaciens</u>	peri	+	+ <sub>2</sub>	+ <sub>2</sub>	+ <sub>2</sub>
262	<u>Acetobacter aceti</u>	peri	+	+	+ <sub>2</sub>	+ <sub>2</sub>
263	<u>Acetobacter oxydans</u>	-	+	+ <sub>2</sub>	+ <sub>2</sub>	+ <sub>2</sub>
277	<u>Acetobacter rancens</u>	-	+ <sub>2</sub>	+	+	+ <sub>2</sub>
339		mono	-	+	+	+

KEY:

- nonmotile or no change of indicator
- + acid reaction of indicator in one day

The subscripts indicate the number of days required to change the indicator.

ously in alcohol media with the peritrichous Acetobacter RH 262 and other variously flagellated organisms in the collection. The carbohydrate base described above was used, to which was added ethanol to give a final concentration of 1, 3 and 5% by volume. The results are shown in Table XVIII.

The criterion for the separation of Acetobacter from Pseudomonas by acid production in high concentration of ethanol is not unique for it appears that other genera are also able to produce acidity in the above medium.

#### UNIT B SUBUNIT I GROUP 2

The second Group of Subunit I was comprised of 13 polar multitrichous organisms. The majority produced a water soluble pigment. A few of the pigmented strains produced a chloroform soluble pigment. Table XIX shows the reactions of these polar multitrichous Pseudomonas strains.

TABLE XIX

## POLAR MULTITRICHOUS PSEUDOMONADS\*

RH numbers	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	citrate	urea	hydrogen sulfide	nitrate	H <sub>2</sub> O sol. pigment	gelatin
199, 200, 257	+	-	-	-	+	+	+	+	-	-	-	-	-
5, 153, 193, 197, 213 280, 298, 302, 355, 384	+	-	-	-	+	+	+	+	-	-	-	+	-

\*The indol, methyl red and Voges-Proskauer tests were negative.

The relationship of these polar multitrichous pseudomonads to

Pseudomonas aeruginosa is very striking. The Pseudomonas aeruginosa strains reviewed in Table XX were true denitrifiers whereas the polar multitrichous pseudomonads do not reduce nitrates.

Pseudomonas syncyanea (Ehrenberg) Migula, (Vibrio syncyaneus, Ehrenberg), was described by Migula (1887) as a water soluble pigment producer. A drawing shows polar multitrichous flagellation. No statement is made concerning carbohydrate utilization. This name is retained in Bergey's Manual of Determinative Bacteriology, 6th edition (1948 page 92).

#### UNIT B SUBUNIT I GROUP 3

This third Group is represented by but one organism, RH 207. It's flagellation was somewhat unusual for it has short flagella with a slight curvature resembling lophotrichous forms.

#### UNIT B SUBUNIT I GROUP 4

The fourth group includes the better known pseudomonads. The blue-green water and chloroform soluble pigmented Pseudomonas aeruginosa is easily identified. Jordan (1899) did not exclude apyocyanogenic strains from consideration as P. aeruginosa. He suggested, without offering convincing evidence, that the difference between Pseudomonas fluorescens and apyocyanogenic strains of P. aeruginosa was one of temperature optima. Many workers, Sandiford (1937), Gaby (1946), and Christie (1948), since Jordan's report have continued to consider P. fluorescens a modified or degenerate form of P. aeruginosa.

There have been several serological reports, Christie (1948), Mayr-Harting (1948), Munoz et al. (1949) and van den Ende (1952), concerned

with the study of pseudomonads but the taxonomic significance of the work is difficult to interpret.

Seleen and Stark (1943) and Haynes (1951) appear to agree that P. aeruginosa will grow at high temperatures, 37-42°C, while P. fluorescens and most other fluorescent bacteria are unable to do so. Haynes found that the ability of P. aeruginosa to grow at 42°C correlated with oxidation of potassium gluconate and slime production.

The first 54 organisms listed in Table XX are considered to be Pseudomonas aeruginosa. No attempt was made to differentiate aeruginosa from fluorescens. These organisms have the following consistent physiological pattern:

dextrose	+	citrate	+ (rarely -)
lactose	-	indol	-
sucrose	-	methyl red	-
maltose	-	Voges-Proskauer	-
mannose	+	urea	-
xylose	+	nitrate	0
arabinose	+		

The carbohydrate pattern is in agreement with the recent report by Liu (1953). The production of a water soluble pigment and liquefaction of gelatin are highly variable characteristics.

This physiological pattern will be recognized as having considerable value in identifying Pseudomonas aeruginosa whether these organisms appear with or without a water soluble pigment. The significance of the above carbohydrate pattern for Pseudomonas will become appreciated when it is realized that it is a response in an organic nitrogen medium. The carbohydrate pattern response established above was reproducible for it was repeated with many strains and with new batches of media many times. The medium

TABLE XX

## PSEUDOMONADACEAE

RH numbers	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	citrate	H <sub>2</sub> S	nitrate	H <sub>2</sub> O sol. pigm't.	gelatin
55, 56, 57, 58, 59, 111, 112, 113, 123, 141, 142, 143, 149, 187, 189, 190, 208	+	-	-	-	+	+	+	+	-	⊕	+	-
1, 3, 122, 144, 145, 147, 148, 150, 151, 156, 161, 188, 191, 192, 195, 196, 202, 209, 210, 217, 218, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428	+	-	-	-	+	+	+	+	-	⊕	+	+
146, 242	+	-	-	-	+	+	+	+	-	⊕	-	+
429	+	-	-	-	+	+	+	+	-	⊕	-	-
157	+	-	-	-	+	+	+	-	-	⊕	-	-
2*	+	-	-	-	+	+	+	+	-	-	+	-
341	+	-	-	-	+	+	+	+	-	-	+	+
66, 204	+	-	-	+	+	+	-	+	-	⊕	-	-
203	+	-	-	+	+	+	-	+	-	+	-	-
129	+	-	-	+	+	-	-	-	-	-	-	+
364 (flesh colored colonies)	+	-	-	+	-	-	-	-	+	-	-	+
198	+	-	-	+	-	-	-	+	-	+	-	-
163	+	-	-	-	-	-	-	+	-	-	-	-
313, 314	+	-	-	-	-	-	-	+	-	-	+	-
300	+	-	-	-	-	-	-	+	-	-	+	+
89, 95	+	-	-	-	-	-	-	-	-	⊕	-	-

Note: The indol, methyl red, Voges-Proskauer and urea tests were negative.

\*Biochemical pattern same as polar multitrichous organisms in Table XIX.

introduced here, in contrast to the inorganic medium, also has the distinct advantage of differentiating oxidizers from fermenters so that there can be no mistake of placing a polar flagellated fermenter or even other fermenters in the genus Pseudomonas.

The citrate reaction was of greater value in this monotrichous group than in the Alcaligenes group for it gave a very decisive change of indicator. Nitrate was very characteristically reduced to nitrogen gas for the Pseudomonas aeruginosa strains. Gas production was confirmed by anaerobic growth studies. This phenomenon was elaborated earlier in the discussion.

The latter 13 organisms in Table XX could be assigned species epithets in the genus Pseudomonas but should perhaps await further study and isolation of more strains before their orientation becomes clear. Would it be more correct to assign organism RH 364 to the genus Xanthomonas rather than Pseudomonas? No attempt was made to establish relationship of these organisms to previously described organisms. Many of these unmistakable pseudomonads did not reduce nitrate to gas but appeared to stop at nitrite or could not reduce nitrate at all.

#### UNIT B SUBUNIT I GROUP 5

The fifth and last Group of Subunit I concerns six nonmotile organisms. Two were labeled Acetobacter (RH 263 and 277). Organism RH 360 with yellow water insoluble pigment could probably be classified, according to Bergey's Manual of Determinative Bacteriology, 6th edition (1949), as a nonmotile organism in the genus Pseudomonas, Xanthomonas or Flavobacterium. The nonmotile organism RH 61 was very interesting to have encountered since ~~it had all the characteristics of Pseudomonas aeruginosa save for motility~~

and flagellation.

#### UNIT B SUBUNIT II

The ten organisms in Subunit II are listed in Table XXI. They are recognized by their ability to oxidize dextrose and sucrose but not lactose. The peritrichous Group 1 in this Subunit II may be members of the genus Agrobacterium, however RH 318 may be a Flavobacterium since it produces a yellow pigment. The remaining nonmotile and polar flagellated organisms may become sucrose positive species in the genus Pseudomonas.

Vibrio cuneata n. sp. was described by Gray and Thornton (1928) as a curved rod with 1 to 5 polar flagella. Drawings indicate polar multitrichous flagellation. Bergey's Manual of Determinative Bacteriology, 6th edition (1948 page 199) retains this organism in the genus Vibrio. This organism was described (see History, Gray and Thornton, 1928) as not producing acid from carbohydrates. Vibrio cuneatus, RH 248, deposited by Gray and Thornton in the American Type Culture Collection, had polar multitrichous flagella but produced oxidative acidity in dextrose and other carbohydrates as shown in Table XXI.

#### UNIT B SUBUNIT III

Organisms in Subunit III oxidize dextrose and lactose but not sucrose. The organisms and their reactions are listed in Table XXII. The entire subunit might be included in the genus Pseudomonas.

The urea reaction for the monotrichous organisms was generally very weak. The positive urea reactions in the table indicate changes of indicator seen in 48 hours. Many times a positive reaction was seen in three days.

TABLE XXI

## UNIT B SUBUNIT II\*

RH number	flagellation												
		dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	citrate	urea	nitrate	H <sub>2</sub> O sol. pigment	gelatin
Group 1													
318 <sub>a</sub>	peritrichous	+	-	+	+	+	-	-	-	-	-	-	+
283	peritrichous	+	-	+	+	+	+	+	-	+	-	-	-
246	peritrichous	+	-	+	+	+	+	+	-	-	+	-	-
177	peritrichous	+	-	+	+	+	+	+	-	-	-	-	-
176	peritrichous	+	-	+	+	+	+	+	-	+	+	-	-
Groups 2, 3, and 4													
248	polar multi	+	-	+	-	+	+	+	+	-	+	+	+
443, 444	monotrichous	+	-	+	+	+	+	+	-	-	+	-	-
272	monotrichous	+	-	+	+	+	+	-	+	-	+	-	-
363	-	+	-	+	-	+	+	+	+	-	-	+	-

## KEY:

\*The indol, methyl red, Voges-Proskauer and hydrogen sulfide tests were negative

a yellow water insoluble pigment



Group 3 includes the nonmotile Bacterium anitratum described by Schaub et al. (1948), Stuart et al. (1949), Ferguson et al. (1950) and Brooke (1951). This is a very homogenous aerobic group not to be confused with the paracolon bacilli which ferment dextrose. Schaub (1948) observed that carbohydrates were oxidized only in the presence of air and that this differed from the fermentation seen among the Enterobacteriaceae. Furthermore a 10% lactose agar slant is not essential to demonstrate acid production since it is readily seen on the 1% lactose medium described. Brooke's (1951) negative lactose finding emphasizes again the importance of using a more suitable medium.

Contrary to Schaub's (1948) finding, a positive methyl red test was not observed. Brooke (1951) found 84 of his 86 strains gave a negative methyl red test.

Balamuth's (1951) organism NRS-11, RH 432, should not be considered an "anaerogenic paracolon bacillus". Strain RH 10 was unusual since it gave a positive Rustigian-Stuart urea test. Schaub found Bacterium anitratum gives a positive Christensen's urea agar test. Brooke also found urea positive strains. Gelatin was positive in but one instance, RH 168, however RH 10 was originally described as giving a positive gelatin reaction. The strains RH 16 and 168 cleared milk agar. The following ten strains listed in Group 3 of Table XXII are considered to be Bacterium anitratum, RH 11, 16, 24, 130, 159, 410, 411, 432, 168 and 10.

#### UNIT B SUBUNIT IV

This last Subunit of Unit B was established on the basis of having

TABLE XXII

## UNIT B SUBUNIT III

RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	citrate	urea	nitrate	H <sub>2</sub> O sol. pigm't.	gelatin
Group 1													
154	polar multi	+	+	-	+	+	+	+	+	-	-	+	-
Group 2													
155, 295, 296, 312	mono	+	+	-	+	+	+	+	+	-	-	-	-
284, 308, 309, 310 311	mono	+	+	-	+	+	+	+	+	+	-	-	-
259	?mono	+	+	-	-	+	+	+	+	+	-	-	-
266	mono	+	+	-	+	+	+	+	+	-	⊕	-	+
Group 3													
11, 16, 24, 130, 159, 410, 411, 432	-	+	+	-	-	+	+	+	+	-	-	-	-
168	-	+	+	-	-	+	+	+	+	-	-	-	+
10	-	+	+	-	-	+	+	+	+	+	-	-	-

Note: The indol, methyl red, Voges-Proskauer and hydrogen sulfide tests were negative.

studied two organisms which oxidized dextrose, lactose and sucrose. These polar flagellated organisms can be given species recognition in the genus Pseudomonas. The reactions of these two organisms, RH 307 and 375, can be seen in Tables II and III.

#### UNIT C

The 39 organisms in Unit C are quite different than the previously considered Units A and B. Unit C is comprised of facultative organisms which ferment dextrose, i.e. they metabolize dextrose in the presence or absence of air. The organisms of this Unit are divided into five Subunits on the basis of response in dextrose and lactose. The basis for the differentiation is seen in Table III. It was not the purpose of this work to consider this Unit in detail, however cursory observations were made to elucidate the fermentative and oxidative spectrum of organisms.

Organism RH 383 ferments dextrose without gas production and oxidizes lactose. The taxonomic status of this organism is not certain but perhaps there is some relationship among the anaerogenic paracolon bacilli.

Two Shigella, RH 436 and 437, madampensis and sonne respectively, were studied which fermented both dextrose and lactose.

Organisms RH 174, 224 and 228 were included here only because they were sent to the laboratory for study labeled as Alcaligenes, an obvious misnomer since they ferment dextrose. It is indeed unfortunate that the American Type Culture Collection and the National Collection of Type Cultures continue to disseminate and carry these organisms as Alcaligenes.

Three peritrichous organisms, RH 304, 344 and 433, one of which

was an authentic paracolon bacillus, were studied which fermented dextrose with gas and oxidized lactose.

Among the genera included in Unit C which fermented dextrose with acid and gas were Salmonella, Proteus, and Aeromonas. Aeromonas is readily distinguished by its monotrichous flagellation. The detailed reactions of the Aeromonas strains studied are given in Table XXIII. These organisms are included in the genus Pseudomonas in Bergey's Manual of Determinative Bacteriology 6th ed. (1948), but since they ferment dextrose the genus Aeromonas seems more suitable. The validity of this genus is reemphasized by Miles and Miles (1951). Peritrichously flagellated individuals are shown to occur, Leifson and Hugh (1953), in early growth phases with only single polar flagella in later stages. Aeromonas liquefaciens strain L418 was sent by J. Kluyver (RH 254) and Ellen M. Miles (RH 271). Both sources of this strain indicated the culture to be monotrichous, see Miles and Miles (1951). In our laboratory this organism was found to be peritrichous. More recently Miles and Kluyver have confirmed the peritrichous flagellation (personal communication 1952). The genus Aeromonas appears to be related to the peritrichously flagellated family Enterobacteriaceae since Aeromonas ferments carbohydrates and produces peritrichous flagella in young cultures.

TABLE XXIII

## THE GENUS AEROMONAS

RH number	flagellation	dextrose	lactose	sucrose	maltose	mannose	xylose	arabinose	indol	methyl red	Voges-Proskauer	citrate	nitrate	gelatin
250, 251, 289, 335, 343, 349, 353	mono	+	-	+	+	+	-	+	+	-	+	+	+	+
252	mono	+	-	+	+	+	-	+	+	-	+	-	+	+
253	mono	+	-	+	+	+	-	-	+	-	+	+	+	+
255	mono	+	-	+	+	+	-	+	-	-	+	+	+	+
292	mono	+	-	-	+	+	-	+	+	-	+	+	+	+
320	mono	+	-	-	+	+	-	-	-	-	+	+	+	+
254*	PERI	+	-	+	+	+	+	+	-	+	+	+	+	+
271*	PERI	+	-	+	+	+	+	+	-	+	+	+	+	+

## KEY:

\* The peritrichous organisms RH 254 and 271 are included here only because Miles and Miles (1951) considered them authentic strains of Aeromonas. Kluyver (personal communication, 1952) has confirmed the peritrichous flagellation of RH 254.

The urease test for these organisms was negative.  
Hydrogen sulfide tests were negative on Kliglers Iron Agar.  
Water soluble pigment was not produced.

## CHAPTER VII

### SUMMARY

1. The history of the genus Alcaligenes has been reviewed and an extensive bibliography presented.
2. Strains of Alcaligenes and related organisms were isolated and accumulated from many laboratories and collections. Their morphological and physiological characteristics are recorded.
3. A technique is presented which differentiates organisms on the basis of carbohydrate utilization. Three units were formed, among the organisms studied, on the basis of metabolism of dextrose, they are: (A) no acidity produced, (B) oxidative acidity produced and (C) fermentative acidity produced. Fermentation is described as a process whereby an organism can obtain energy from carbohydrates in the absence of oxygen.
4. One hundred and twenty organisms were studied which did not produce acidity in dextrose media. Bacteriologists have been inclined to place most of these organisms in the genus Alcaligenes even though they are not homogeneous in respect to morphology and physiology. These organisms were divided into groups on the basis of flagellar morphology.
5. Applying the above concepts, the genus Alcaligenes is limited and defined as follows: Gram negative rods. Peritrichous or nonmotile.

Growth on agar slants nonchromogenic to yellow. The pigment does not diffuse through the agar. (Eldering and Kendrick, 1952, do not describe parapertussis as producing a pigment but prefer to call it a "browning of peptone medium".) Does not oxidize or ferment dextrose, lactose, sucrose, maltose, mannose, xylose or arabinose. Indol, methyl red and Voges-Proskauer tests negative. Hydrogen sulfide test negative on Kligler's iron agar. The species are differentiated in Table XXIV.

TABLE XXIV DIFFERENTIAL CHARACTERISTICS OF THE ALCALIGENES SPECIES			
	flagella	urea	nitrate
<u>A. bronchicanis</u>	+	+	+
<u>A. faecalis</u>	+	-	±
<u>A. denitrificans</u>	+	-	⊖
<u>A. viscosus</u>	-	-	-
<u>A. parapertussis</u>	-	+	-

\*occasionally negative

6. Three groups of organisms are now clearly excluded from the genus Alcaligenes. They are placed in three separate genera. (A) Xanthomonas maltophilia sp. nov. was created for polar multitrichous dextrose nonoxidizers which produce oxidative acidity from maltose. (B) The designation Lophobacter faecalis gen. et sp. nov. is proposed for lophotrichous nonoxidizing gram

negative rods. These organisms have been shown to have a morphological and physiological relationship to Spirillum virginianum. (C) Pseudomonas alcaligenes is retained for the monotrichous nonoxidizing gram negative rods.

7. There are four groups of dextrose oxidizers which warrant emphasis here. (A) There appears to be no objection to the use of Pseudomonas syncyanea to represent the polar multitrichous water soluble pigment producers listed in Table XIX. (B) Pseudomonas aeruginosa represents strains with any combination of pyocyanine or fluorescein or even without pigment. These organisms are described as having a very definite morphological and physiological pattern. (C) The genus designation Agrobacterium should probably be reserved for peritrichous rods which oxidize dextrose and sucrose but not lactose. (D) The biochemical activity of Bacterium anitratum is shown to be a homogenous group of aerobic organisms when limited to dextrose and lactose oxidizers as shown in group 3 in Table XXII.

8. The genus designation Aeromonas is appropriate for monotrichous gram negative rods which ferment dextrose with the production of acid and gas. They are organisms which show a morphological relationship between the families Pseudomonadaceae and Enterobacteriaceae.

9. The designations Alcaligenes bookeri, recti, marshalli and metalcaligenes should become nomina dubia since the application of these epithets is uncertain.



## CHAPTER VIII

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APPROVAL SHEET

The dissertation submitted by Rudolph Hugh has been read and approved by three members of the Department of Microbiology and four faculty members outside of the Department of Microbiology.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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Date

Einar Leifson

Signature of Sponsor