SPORE-BEARING BACTERIA IN MILK

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It has been pointed out by a number of observers (Flügge, 1904; Ford and Pryor, 1904) that milk heated to any temperature above 60°C, if kept in a warm place, shows an excessive development of spore-bearing bacteria which are ordinarily inhibited by the lactic acid bacteria universally present. Hueppe (1884) was possibly the first to call attention to the presence of aerobic spore-bearing forms in milk but it is impossible to say now what his *Bacillus butyricus* (an aerobe) really was. Subsequently Loeffler (1887) described an organism from boiled milk which had been allowed to clot, under the name *Bacillus lactis albus*, now known as *Bacillus albolactus* Migula. Considerably later Flügge (1894) took up the question at some length and described eleven different species found in boiled milk and to them he ascribed an etiological rôle in the summer diarrhoea of infants. Several of the organisms described by Flügge are now considered identical with such common saprophytes as *Bacillus vulgatus* and *Bacillus mesentericus* while others can be identified with difficulty or not at all, as their originals have been lost. During the past three years we have worked out the morphological and cultural reactions of 250 spore-bearing bacteria obtained from raw milk and from milk subjected to various temperatures. The two most common species proved to be *Bacillus cereus* of Frankland and *Bacillus subtilis* of Cohn. In this differentiation we follow Chester who has given us a definite and clear conception of Cohn's species and has taken up at length the somewhat involved discussion concerning the two organisms. As a result of his work Chester decided that the real *Bacillus subtilis* of Cohn is one of the smallest of the spore-bearing species, forms central or slightly excentric spores which have a characteristic appearance and gives definite cultural reactions. The reactions as outlined by Chester we are able to confirm in the main but we disagree absolutely from him in his contention that this species is identical with *Bacillus vulgatus*, (*B. mesentericus vulgatus*) the old fashioned "potato bacillus."

The cultures identified by us as *Bacillus subtilis* corresponded in all particulars to a culture sent us several years ago by Chester and kept in the laboratory since then. The particular points by which *Bacillus subtilis* may be differentiated from *Bacillus vulgatus* are the development on glucose litmus agar where it forms a dry hard warty growth made up of dense masses of material clinging firmly to the medium, in which may be observed numerous blebs or blisters containing milky fluid, and on Loeffler's blood serum where a similar growth appears, often however with a distinct red color. On both glucose litmus agar and blood serum *Bacillus vulgatus* develops as a soft wrinkled friable mass easily broken and lifted from its substratum. On potato the *subtilis* differs from the *vulgatus*. The former produces at first a rather dense whitish or greyish mass often showing blebs similar to those on agar and blood serum, a distinct red line appearing in the potato a little distance from the growth, from which characteristic the name *Bacillus subtilis-ruber* is frequently employed. After 48 to 72 hours a wrinkling appears, the growth later becoming moist and homogeneous. *B. vulgatus* produces a wrinkled growth from the start, this becoming extremely abundant in 3 to 4 days and frequently assuming a decided pink color. The differences between the two species are somewhat difficult to describe but when potato cultures of the organisms are placed side by side the points of differentiation become clear and definite. In general the *subtilis* cultures are dry and hard on solid media and produce firm tenacious scums on fluids, while the *vulgatus* cultures are soft and mealy and their scums friable and easily broken. On a morphological basis it is extremely puzzling to attempt the differentiation of the two types. In general the rods of *Bacillus vulgatus* are longer and thinner than those of *Bacillus subtilis* while the spores are flatter and bulge the organism only a little if at all.

This view of *Bacillus subtilis* of Cohn and the interpretation put on Cohn's work by Chester is not entirely accepted by bacteriologists but we feel convinced of its correctness except in regard to the differentiation from *Bacillus vulgatus*

already mentioned. The organisms frequently regarded as *Bacillus subtilis* which are characterized by their greater size, their soft mealy growths on hard media, and their thick friable scums we agree with Chester in referring to the "cereus" group the principal type of which, *Bacillus cereus*, was first described by the Franklands. There are two strains of *Bacillus cereus* differentiated by their action on saccharose but it does not seem wise at the present time to divide the species. Our identification of *Bacillus cereus* rests upon Chester's work and upon cultures sent us by him several years ago. In addition a number of strains of *Bacillus cereus* have been received from American laboratories and from the Kral collection in Vienna, all of them agreeing with Chester's in their reactions and thus furnishing us a distinct type by means of which our own strains were identified. *Bacillus cereus* is the most widely distributed aerobic spore-bearing organism in nature in Baltimore and vicinity, as it seems to be in other localities, and possibly has more synonyms than any other species. With these types of *Bacillus subtilis* and *Bacillus cereus* clearly outlined the task of identifying the other spore-bearing organisms became somewhat simpler. *Bacillus vulgatus* was soon found so frequently as to enable us to recognize it without difficulty. One strain of this organism was obtained from the Winslow collection in New York. When freshly isolated the *vulgatus* is very characteristic .and differs entirely from other species. The strains isolated in Baltimore were identical with the organisms found in Montreal several years ago and regarded there as *Bacillus vulgatus* and give reactions ascribed to the widely distributed "potato bacillus." *Bacillus mesentericus* (*B. mesentericus fuscus*) was recognized by its morphology and its cultural reactions. In this species we follow Chester. In one instance we obtained a stock culture of *Bacillus mesentericus* which gave the correct reactions as outlined by Chester, this culture coming from the laboratory of hygiene of the University of Pennsylvania. *Bacillus pumilus* of Gottheil we do not regard as a distinct species. *Bacillus aterrimus* (*B. mesentericus niger*) was identified by its production of a black or grey-black pigment, its cultural reactions resembling those of

B. vulgatus. Another organism producing a black pigment and evidently belonging to the mesentericus group was sent us by Winslow as *Bacillus lactis-niger.* It corresponds culturally to *Bacillus mesentericus.* It was not encountered in our work but is included here for the sake of completeness. The same holds true of the organism described originally as *Bacillus mesentericus-ruber* (properly *B. globigii*) a culture of which was obtained from the Kral collection in Vienna. Evidently this is an extremely rare organism in this country as it was never obtained in Baltimore either from milk or from any other source.

One of the most difficult organisms to identify was a species frequently isolated in Baltimore from milk which after boiling clots and peptonizes. In morphology and in its chief cultural reactions it corresponds closely to *Bacillus cereus* but is differentiated from this species by its acid fermentation of lactose and its coagulation of milk. This organism was evidently first described by Loeffler in 1887 as *Bacillus lactis albus* (*Bacillus albolactus* Migula). We have been unable to obtain a culture of Loeffler's organism but in his original description Loeffler differentiates this species clearly from several other organisms found in milk particularly the ones now known as *Bacillus vulgatus* of Flügge, *Bacillus liodermos* of Flügge, and *Bacillus butyricus* of Hueppe. Since Loeffler was the first to call attention to the presence of an organism in boiled milk which acidifies and clots it and which he differentiated from other spore-bearing bacteria, we feel that similar organisms from boiled milk which correspond to Loeffler's description should be regarded as identical with his species. We therefore propose to utilize the name *Bacillus albolactus* Migula, (synonym *Bacillus lactis albus* Loeffler) for the organisms isolated from the source studied by Loeffler. This organism is undoubtedly isolated from time to time by other bacteriologists and must exist in a number of laboratories. It was apparently described recently by Neide (1904) in Meyer's laboratory as *Bacillus teres. Bacillus albolactus* is, we believe, the common cause of the clotting and peptonization occasionally seen with boiled milk. It is undoubtedly also a contributing

factor to the changes seen in milk pasteurized at lower temperatures, 60° to 65°C., which subsequently develops a bitter taste.

Bacillus mycoides was identified without difficulty by the classical descriptions and by the work of Chester whose conclusions were based upon a culture which was sent him from our laboratory several years ago. The felted growths in the depths of agar are very characteristic and are given by but one other species, *Bacillus ramosus-liquefaciens of* Prausnitz.

For a long time we were in doubt as to the identification of the very large microörganisms which are placed in a heterogeneous group and sometimes called *Bacillus megatherium,* sometimes *Bacillus petasites,* and sometimes *Bacillus tumescens.* The first member of this group was described by De Bary (1884, 1887) whose illustrations are very characteristic. Many of the cultures identified and sent to us as *Bacillus megatherium* differed radically from De Bary's description, and Chester's conclusions in regard to the ill-defined character of the group seemed to be entirely justified. These large organisms were very abundant however and soon resolved themselves into two distinct types. One type agreed with De Bary's original description in all essential particulars and this type agreed also with an isolation of *Bacillus megatherium* by Kellermann sent us from the Winslow col'ection. Two cultures of *Bacillus tumescens* of Zopf agreed closely with this *Bacillus megatherium* and there seems to be no reason to regard it as a distinct species. The other type has almost the same morphology and the same cultural reactions as *Bacillus megatherium* but produces an intense yellow pigment. This type corresponds to the organism recently described as *Bacillus petasites* by Gottheil. All the organisms thus far encountered with the morphology referred to can thus easily be divided into these two main forms. The *Bacillus graveolens* of Gottheil, not the *Bacillus graveolens* of Bordoni-Uffreduzzi, seems to be merely a strain of *Bacillus megatherium* in which the bacilli have a peculiar property of growing in short spirals. It has not been encountered in our work.

The *Simplex-cohaerens* group of Chester proved possibly the

most difficult of all to clarify. Two organisms were originally described by Gottheil as distinct species, regarded by Chester however as practically identical. Strains of *Bacillus simplex* and *Bacillus cohaerens* received by us from Kral were quite different morphologically and while it is evident that we lack many of those pronounced cultural and morphological reactions which render species and groups easy to recognize yet we must not therefore place organisms together which are clearly different. On one occasion we found in milk an organism evidently identical with the strain of *Bacillus cohaerens* received from Kral and corresponding to Gottheil's original description. Subsequently this species was found five times by Dr. Laubach in soil. These organisms gave us a fairly clear idea of the species and its differentiation from *Bacillus simplex* whose description we also give here. This latter description while made from a strain isolated by Gottheil, applies also to a species subsequently found in dust by Dr. Laubach. On two occasions we isolated from milk the species described as *Bacillus fusiformis* by Gottheil. Our isolations were identical with Gottheil's in every particular. Finally on one occasion we obtained a strain with properties practically the same as those of the species described by Flügge as No. XII and now known as *Bacillus terminalis* Migula.

The 250 cultures studied were from 68 samples of milk, 12 of raw milk, 12 of milk pasteurized at 60°C., 32 of milk heated to 85°C., and 12 of boiled milk. These cultures may thus be held to represent so many various conditions in the development of the bacteria of milk as to give an accurate idea of the spore-bearing organisms of milk in Baltimore and they probably represent conditions met with elsewhere. By their combined development in heated milk they give rise to the putrid decomposition so frequently observed. As can be seen from their cultural reactions these organisms are in the majority of instances energetic protein-splitters and in practically every case rapidly dissolve the casein in milk either before or after a preliminary coagulation.

After the various types of spore-bearing organisms were established by the study of 250 cultures from the 68 samples

mentioned above another series of milks, also subjected to various treatments, was investigated with the object of testing the preliminary classification adopted. In this second portion of our work the types previously established were abundantly confirmed but no new organisms were isolated. We believe therefore that the organisms first worked out represent the spore-bearing organisms usually present in Baltimore milk. Our original 250 cultures show the various species in the following proportions.

Baltimore Milk

Bacillus cereus Frankland	124
Bacillus subtilis (Ehrenberg) Cohn	79
Bacillus albolactus Migula	25
Bacillus vulgatus (Flügge) Trevisan (*Bacillus mesentericus vulgatus* Flügge.)	15
Bacillus mesentericus (Flügge) Migula (*Bacillus mesentericus fuscus* Flügge.)	2
Bacillus fusiformis Gottheil	2
Bacillus petasites Gottheil	1
Bacillus cohaerens Gottheil	1
Bacillus terminalis Migula	1

In addition to these the following species were isolated from other sources during the work on milk and made the basis for comparison. We have reason to believe that they may occur in milk, partly from the work of others and partly because they are not infrequent in milk products. We introduce them here for completeness.

Bacillus mycoides Flügge.

Bacillus megatherium De Bary.

Bacillus simplex Gottheil.

Bacillus aterrimus Lehmann & Neumann (*Bacillus mesentericus niger* Lunt).

Bacillus niger Migula (*Bacillus lactis niger* Gorini).

Bacillus globigii Migula. (*Bacillus mesentericus ruber* Globig).

Finally a brief note is added in regard to certain other cul-

tures sent us which we do not regard as entitled to specific rank, namely

Bacillus pumilus Gottheil.
Bacillus graveolens Gottheil.
Bacillus tumescens Zopf.

Bacillus cereus Frankland 1887

This organism was first described by the Franklands in 1887 (Franklands, 1887). It has since been described under a host of names and it is impossible to say how many different species are identical with it. It is the most widely distributed organism of this group in Baltimore, being found abundantly in milk, soil, dust, water, and in the intestinal contents. It is particularly common as a laboratory contamination. The present description applies to cultures received from the Kral collection, from the American Museum, and from a number of American laboratories and to over a hundred of our own isolations.

Morphology. Regular bacilli with homogeneous protoplasm and rounded ends, in young cultures measuring about 0.75 by 2.25 to 4 microns. Many of the organisms show peculiar refractile bodies of various sizes as the cultures get older, presenting a characteristic appearance. The nature of these bodies is not clear as they do not give reactions for starch or volutin. They can usually be differentiated from the beginning spores. On glucose agar the bacilli are thicker and longer measuring 0.75 to 1 by 3 to 6 microns. Here the entire protoplasm of the organism is converted into the bodies mentioned above. They are globular, highly refractile, and are often as thick as the organism. (Figures 25, 26 and 27.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore-formation. Spores are formed early on both plain and glucose agar, often appearing within 24 hours or even in less time. They may be central in position, excentric or even subterminal but the latter location of the spore is rare. The spores are usually wider than the organisms from which they spring

and thus bulge the rods slightly. The free spores retain their protoplasm at the ends for some time, usually in equal amounts. Often, however, the protoplasm is greater at one end than at the other and the spore then has a characteristic appearance like an enlarged mesentericus spore. The free spores are cylindrical, soon shed their protoplasm and measure 0.5 to 0.75 by 1.125 to 1.5 microns.

Agar slant. Abundant, thick, white mealy growth along the line of inoculation sometimes with arborescent edges. In older cultures the growth is much thicker, yellowish white and may show pellucid areas surrounded by more highly refractive patches.

Agar stab. Little growth along line of inoculation but luxuriant surface growth spreading over entire surface of agar and extending to the walls of the tube.

Agar colonies. Round, raised, dense, highly refractive surface colonies. If slight amount of water of condensation be present the colonies may be amoeboid. Under low power the colonies consist of dense central nuclei with spreading peripheries made up of numerous curling and parallel chains. The colonies are soft and easily lifted from the agar. Deep colonies punctiform, stellate or rhizoid. Under the low power they are fuzzy, irregular and may resemble a chestnut burr.

Litmus glucose agar slant. Thick, yellowish-white growth along the line of inoculation and spreading out over entire surface. The medium is acidified and the growth is sometimes distinctly yellow. Typical cultures rapidly decolorize the litmus and then become alkaline and the agar turns deep blue. Occasionally the cultures are less active alkali-producers and the medium remains permanently acid. Such cultures, however, can usually be stimulated to alkali production by plating and they then give characteristic growths.

Glucose agar colonies. Surface colonies round or bizarre, heaped up, with irregular margins, smaller than plain agar colonies. Under low power granular, with dense central nuclei and irregular margins, showing fine parallel strands. Deep colonies small irregular or round. Under low power they consist of dense central nuclei with fine, irregular or parallel strands in the periphery.

Gelatin stab. Uniform growth along entire line of inoculation with a liquefaction also along entire line. The liquefaction becomes cup-shaped or sacculated with a surface scum. It is rapid and frequently in two days the entire gelatin tube is liquefied.

Gelatin colonies. Loosely filamentous colonies with dense, central nuclei and spreading irregular margins, often very thin, edges entire. Gelatin liquefied rapidly.

Broth. Very turbid in 24 hours with no scum except occasionally a slight ring growth. In two days a heavy friable scum is produced which is entirely precipitated within a short time. The medium gradually clears while a heavy flocculent precipitate is deposited.

Peptone. Very turbid in 24 hours. Scum appears usually on the second day and is soon precipitated. It is like that produced in broth but is more friable.

Potato. Thick, white, mealy growth in a few days becoming yellowish or brown with a discoloration of the potato. This brownish growth may become very moist and slimy and is occasionally measley but never vermiform. It never assumes an appearance similar to that seen with cultures of *Bacillus subtilis* or *Bacillus vulgatus.*

Litmus milk. With the majority of cultures peptonization begins immediately and progresses rapidly in three zones. Surface zone is amber, middle zone violet, the lowest zone blue. Peptonization continues until the entire milk tube is converted into an amber fluid with a slight sediment. Milk does not coagulate. With some cultures the three-zone appearance does not show but the milk is gradually changed to a muddy gray-colored fluid. Eventually, however, the same clear amber-colored fluid is produced.

Blood serum. Thick, white, dry, smooth growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl and arm. Friable scum forms which is soon precipitated. Turbidity gradually disappears and a flocculent precipitate is deposited. Reaction highly acid.

Saccharose. Abundant growth in bowl and arm. Filmy and

friable scum forms which is soon precipitated. In many cultures the reaction is acid. Reaction alkaline in the majority of cultures.

Lactose. Turbidity in bowl with scum formation. Arm clear. Reaction alkaline.

Thermal death point. The spores survive steaming one hour in the Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus albolactus Migula 1900

This organism was apparently first obtained in pure culture in 1887 by Loeffler who found it in boiled milk which had soured and clotted and who named it *Bacillus lactis-albus.* It is possibly identical with *Bacillus corrugatus* Migula (1900) (Bacillus No. II Flügge), with *Bacillus bernensis* Lehmann and Neumann, (1901) and with the organism described recently by Neide as *Bacillus teres* which was also obtained from boiled milk which had subsequently soured. It is common in boiled milk in Baltimore and produces the souring, clotting, and subsequent peptonization seen so frequently in this material.

Morphology. These organisms are identical morphologically with *Bacillus cereus.* In young cultures 6 to 24 hours old, on plain agar, they have round ends and measure 0.5 to 0.75 by 2.25 to 4 microns. The protoplasm may be homogeneous or may show globular bodies of various dimensions. On glucose agar the globular bodies are much more abundant and give the organism a characteristic appearance. Here the rods measure 0.75 to 1 by 2.5 to 4 microns. (Figs. 28, 29, and 30.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed readily on plain and on glucose agar. They are abundant in 24 to 48 hours and have the same appearance as the spores of *Bacillus cereus.* They are usually central or slightly excentric and a trifle wider than the organisms from which they spring thus bulging the rods somewhat. The free spores may retain equal or unequal bits of

protoplasm at the ends and thus have a characteristic appearance. They are oval to cylindrical and measure 0.5 to 0.75 by 1.5 to 2.125 microns. The spores are frequently seen in pairs attached by their protoplasmic remnants, and also sometimes in chains.

Agar slant. Luxuriant, thick, white growth with a smooth and glistening surface, spreading over the entire surface of the agar. Some cultures show a delicate transverse wrinkling.

Agar stab. Fine, slightly arborescent growth along line of inoculation. Thick, white, wrinkled surface growth.

Agar colonies. Surface colonies thick, raised, round or bizarre, frequently showing dense, central nuclei. Under low power granular with dense, central nuclei and spreading peripheries made up of curved parallel strands. Deep colonies small, round or irregular. Under low power irregular, mossy, with irregular fuzzy margins.

Litmus glucose agar slant. Thick, yellowish-white, moist growth, spreading over the entire agar and wrinkling slightly at the base when the culture is very active. Reaction in medium acid in first few days but gradually alkali is produced and the agar turns dark blue.

Litmus glucose agar colonies. Surface colonies thin, translucent, somewhat smaller than plain agar colonies. Under low power granular with thin peripheries made up of curling parallel strands. Deep colonies round or irregular. Under low power irregular, mossy with irregular, fuzzy margins. Medium first acidified and then made alkaline.

Gelatin colonies. Surface colonies round, spreading concentrically and composed of a central loose mass of filaments denser than the surrounding zone. Deep colonies are composed of spherical masses of loose filaments with irregular, mossy or bristling margins. Rapid liquefaction.

Gelatin stab. Growth along line of puncture with a rapid cup-shaped liquefaction and scum production.

Broth. Turbidity with ring growth in 24 hours and scum formation in 2 to 3 days. Scum quickly precipitated.

Peptone. Turbidity with scum formation on the second day. Scum usually persists.

Potato. Thick, white, moist growth later becoming yellowish brown. Never wrinkled or vermiform, rarely measley. Medium discolored.

Litmus milk. Acid production and coagulation, usually within 24 hours. The coagulum is at first firm but gradually undergoes peptonization, and is usually completely dissolved at the end of three weeks. Odor distinctly faecal and very disagreeable, with a suggestion of indol.

Blood serum. Thick, white growth. No liquefaction.

Fermentation tubes. Glucose. Abundant growth in bowl extending up into closed arm which becomes turbid. Flocculent precipitate forms but usually no scum. Reaction highly acid.

Saccharose. Turbidity in bulb extending up into the closed arm. Flocculent precipitate. No scum. Reaction highly acid.

Lactose. Turbidity in bowl extending up into the closed arm. No precipitate but usually a thick scum is formed. Reaction highly acid.

Thermal death point. Organisms have survived 1 hour in the Arnold sterilizer and autoclaving to 15 pounds pressure. Killed by 16 pounds pressure.

Bacillus subtilis (Ehrenberg) Cohn

Synonyms. Vibrio subtilis Ehrenberg 1838; *Bacillus subtilis* Cohn 1872; *Bacillus subtilis* (Ehrenberg) Cohn, Migula 1900.

Considerable difference of opinion exists as to the correct interpretation of the somewhat puzzling literature concerning this organism. In this paper we have followed the views of Chester who has identified a number of organisms isolated in this country as the real *Bacillus subtilis* of Cohn, and who sent one of his isolations to our laboratory several years ago. It is one of the commonest organisms in milk, soil, dust and water. In morphology it is one of the smallest of the aerobic spore-bearing bacteria and is thus easily distinguished from *Bacillus cereus* with which it is most often confused.

Morphology. Small, thin, homogeneous bacilli measuring 0.375 by 1.5 to 2.5 microns in 24 hour agar cultures. Some-

what thicker and longer on glucose agar measuring 0.5 by 1.5 to 4 microns. Does not usually form threads on this medium. (Figures 4 and 5.)

Motility. Sluggishly motile in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing within 24 hours on plain and glucose agar. They arise in the center or towards one end of the rods and are slightly greater in diameter than the rods, thus causing a distinct bulging. The free spores may retain bits of protoplasm at each end, often unequal in amount, giving the spore a characteristic appearance. Such spores measure about 0.5 by 0.875 microns. The spores rapidly lose their protoplasm, become more oval and measure about 0.5 by 0.75 microns.

Agar slant. Weakly refractive, glassy, membranous growth along line of inoculation, later spreading out over entire surface of agar. The surface is usually dry and hard, but in old cultures it becomes soft and smeary, but is always firmly attached to the agar from which it cannot be scraped off.

Agar stab. Little growth along the line of inoculation but a spreading, dry, membranous growth on the surface of the agar, extending to the wall of the tube.

Agar colonies. Surface colonies weakly refractive, spreading concentrically or in amoeboid fashion from small dense nuclei. Under the low power edges may be complete or finely crenate. If water of condensation be present one or two colonies frequently overgrow the entire plate. Under the low power the colonies are homogeneous and granular or irregular and gyrose. The deep colonies are punctiform and under the lower power lichen-like with irregular margins myceleoid in character. The colonies are usually membranous dry, hard, and glassy, and can be separated from the agar only with great difficulty.

Glucose litmus agar slant. Highly refractive growth verrucose or vesicular, with milky liquid in vesicles, not spreading. Parts of growth show distinct red pigment. Acid is produced in 24 hours, but is replaced by alkali in about ten days, medium turning deep blue.

Litmus glucose agar colonies. Irregular, spreading, bizarre surface colonies, usually more luxuriant than plain agar colonies. Under low power, irregular with entire edges or fuzzy, with myceleoid outgrowths from dense central nuclei. Deep colonies slightly irregular or punctiform. Under low power irregular myceleoid with filamentous edges. Medium first acidified then made alkaline.

Gelatin stab. Slow growth along line of inoculation and rather slow cup-shaped, surface liquefaction with scum production.

Gelatin colonies. Surface colonies round, homogeneous, spreading, thin and granular. Deep colonies yellowish brown, highly refractive. Under low power granular. Colonies may also show dense central nuclei and thin myceleoid filamentous growth extending in every direction through the medium. Gelatin liquefied.

Broth. Single isolated pellicles appear on the surface in 24 hours. In 48 hours these unite to form a thin branching scum, which gradually becomes more dense and tough. Medium grows turbid in first 24 hours, but later clears. Scum is precipitated as a whole in about ten days. This manner of scum formation is characteristic of *Bacillus subtilis.*

Peptone. Turbidity in the first 24 hours and gradual clearing with a flocculent precipitate. Scum on the surface formed in the same manner as on broth, but not so dense or tough. The pellicles often show chains and branching figures. Frequently the scum has a delicate pink color after about five days' growth.

Potato. Growth on potato characteristic. It is luxuriant and warty, having the appearance of many large and small dew drops scattered along the line of inoculation. In 48 hours a pink pigment collects on top of this growth and persists. In older cultures a decided rose-red line in the substance of the potato marks the limit of the growth. In ten days the vesicles dry down and only a reddish-brown dry growth remains on the discolored medium. Later the growth is moist and sticky.

Litmus milk. No change in 24 hours and sometimes none in 48 hours except that the milk becomes more alkaline. In three days the medium begins to clear from the surface, the deeper

parts remaining unchanged. Clearing progresses slowly, the supernatant fluid persisting as a grayish, pinkish or yellowish muddy medium. After a month at room temperature the medium may become very alkaline and turn deep blue-purple. Milk never coagulates.

Blood serum. Vesicular, dew-drop growth with pink color often very marked, in 24 hours. Vesicles dry down eventually leaving a hard wrinkled growth. Medium is not liquefied.

Fermentation tubes. Glucose. Turbidity in bowl and arm. Scum formation like that seen in broth. Highly acid.

Saccharose. Turbidity in bowl and arm with a fragile scum forming from pellicles in about two days. Acid production but not so marked as in glucose.

Lactose. Turbidity in bowl and extending up in the arm to the level of the medium in the bowl. Rest of the arm clear. Dense tough scum. Reaction alkaline.

Thermal death point. Spores survive steaming $1\frac{1}{4}$ hours in the Arnold sterilizer. Survive autoclaving up to and including 19 pounds pressure but usually destroyed by 20 pounds pressure.

Bacillus vulgatus (Flügge) Trevisan.

Synonomy. Bacillus mesentericus vu'gatus Flügge 1886; *Bacillus vulgatus* Trevisan 1889; *Bacillus vulgatus* Eisenberg 1891; *Bacillus vulgatus* (Flügge) Migula 1900.

This organism was first described by Flügge in 1886 (Flügge, 1886) and is commonly known as the "potato bacillus." According to Chester it is identical with *Bacillus subtilis.* By the use of glucose agar and blood serum and by the careful observation of the cultural reactions, particularly in broth and on potato the species is easily separated from this organism. It is fairly common in Baltimore but by no means as frequent an isolation as are many of the other spore-bearers.

Morphology. Small homogeneous organisms usually distinctly larger than *Bacillus subtilis,* measuring 0.5 by 2 to 3 microns. Occasionally short forms 1.125 and long forms measuring 4 microns are seen on plain agar. On glucose agar the organisms

are thicker and much longer measuring often nearly 0.75 microns in thickness and 5 microns in length. (Figures 6 and 7.)

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed early appearing in 24 hours on plain and glucose agar. They arise in the center or towards one end of the rods but do not ordinarily bulge the rods appreciably. When free they are elongated and flattened and retain tags of protoplasm at each end. At times the protoplasm at one end is greater in amount then at the other. Such spores measure about 0.5 by 1.125 microns. As they lose their protoplasm they become cylindrical measuring about 0.5 by 1 micron. In general the spores are about the same width as the vegetative rods or only very slightly wider.

Agar slant. Moist profuse thick growth on agar, easily lifted or brushed from the surface of the medium with the platinum wire. Growth is usually white or cream white, spreads but little from the line of inoculation and is whitest at the edge where it is heaped up. When water of condensation washes over the agar many small, round colonies develop apart from the main growth. In some strains the agar growth is dry and fine wrinkles develop but the growth can always be lifted from the agar with a platinum loop.

Agar stab. Little growth along line of inoculation but rather dry wrinkled rooty growth spreads over the surface of the agar.

Agar colonies. Surface colonies round, waxy, highly refractive or spreading and amoeboid with greatest refraction at the edge of the advancing growth, where colonies are thickest. Under low power of the microscope edges entire. Deep colonies punctiform, round or elliptical. Under low power they are irregular, brown, slightly granular with entire or fuzzy edges.

Litmus glucose agar slant. Characteristic appearance. Luxuriant dry brown and abundantly wrinkled growth develops within 24 to 48 hours. The medium is acidified. After a few days the growth usually becomes moist and the wrinkles are obliterated while the medium becomes alkaline and turns deep blue.

Litmus glucose agar colonies. Superficial colonies are thick, highly refractive, waxy, with entire edges or spreading with irregular edges. They soon become dry and wrinkled in the center. Under low power opaque with entire edges. Deep colonies are punctiform, round, oval or irregular with crenated margins. Under low power opaque with irregular margins. Medium acidified at first then turned alkaline.

Gelatin. Stab gives cup-shaped and surface liquefaction with heavy scum production.

Gelatin colonies. Colonies round with highly refractive centers occasionally showing beautiful concentric rings. Under the low power the colonies have a granular appearance. Medium liquefied.

Broth. Turbidity within 24 hours and scum formation usually on second day. Medium gradually clears.

Peptone. Turbidity within 24 hours. Thin fragile scum after the lapse of several days. Medium gradually clears.

Potato. Characteristic appearance. Thick, white, gray or pink folds or wrinkles are formed within 24 to 48 hours often covering entire cut surface of potato. Later these folds dry down to a brown, reticulate mass. Potato usually discolored.

Litmus milk. Slight clearing of the milk just beneath the cream layer usually appears in 24 hours. This reaction rapidly intensifies with the production of a clear fluid colored deep Chinese-blue or purple. No acid production or coagulation. As the milk gets older complete peptonization occurs with the formation of a clear amber-colored fluid.

Blood serum. Thin dry abundant growth usually smooth, but sometimes wrinkled and pink. Growth later becomes moist and gives a suggestion of liquefaction.

Fermentation tubes. Glucose. Luxuriant growth in bulb gradually extending into the closed arm. An abundant scum is formed which may be quite wrinkled. Reaction acid.

Saccharose. Growth luxuriant in bowl but scanty in arm. Very thin scum may be formed after several days, but this may be lacking. Reaction varies from slight to marked acidity.

Lactose. Abundant growth in bowl with late scum production. No growth in closed arm. Reaction alkaline

Thermal death point. Organisms survived heating in broth in the Arnold sterilizer for one hour. Survived autoclaving up to and including 19 pounds pressure, but were destroyed by 20 pounds pressure.

Bacillus mesentericus (Flügge) Migula 1900

This organism was first described by Flügge in 1886 (Flügge, 1886) as a species distinct from *Bacillus mesentericus-vulgatus* and named *Bacillus mesentericus-fuscus*. We have isolated a number of organisms which correspond to the description given by Flügge and also by Chester. It is one of the less common of the aerobic spore-bearing bacteria but occurs in milk, soil, dust and water.

Morphology. Organisms about the same in morphology as *Bacillus mesentericus-vulgatus*. On agar cultures in 24 hours they are homogeneous rods measuring 0.5 by 1.5 to 3 microns. Sometimes shorter forms predominate in the cultures, a little over a micron in length. On glucose agar they are thicker and longer measuring 0.75 by 2 to 5 microns, with many long forms measuring 6 to 8 microns in length. (Figures 8 and 9.)

Motility. Active motility, progressive and rotatory, in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form in 24 hours on plain and on glucose agar. By the end of 48 hours they are very abundant. They appear in the center or towards one end of the rods and do not bulge the organism appreciably. The free spores are cylindrical and may retain equal bits of protoplasm at each end or this protoplasm may be unequal in amount giving a characteristic appearance to the spore. They measure about 0.5 by 1.125 microns. They rapidly lose their protoplasm and become slightly more oval, measuring 0.5 by 0.75 microns.

Agar slant. Soft white or cream-white growth somewhat translucent when old, spreading but little from the line of inoculation except in the presence of water of condensation. Easily lifted from the agar. Edges of growth irregular or serrate. Growth does not become dry or wrinkled.

Agar stab. Little growth along line of puncture, luxuriant growth on surface.

Agar Colonies. Superficial colonies round highly refractive with entire edges, or spreading and amoeboid. Under low power opaque with crenated edges. Deep colonies round and regular. Under low power slightly granular with crenated margins.

Litmus glucose agar slant. Thick, abundant, white or cream white to yellow growth spreading along the line of inoculation. Medium first turns acid but as growth becomes older it again becomes deep blue.

Litmus glucose agar colonies. Superficial colonies round, highly refractive, with entire edges or spreading and amoeboid with densest part of the growth along the advancing edge. Under low power of the microscope edges crenated. Deep colonies round or oval and under low power slightly granular with crenated margins. Medium first acidified and then made alkaline.

Gelatin stab. Cup-shaped or surface liquefaction and scum production.

Gelatin colonies. Colonies dense with liquefaction centers and granular ring at the edges of a cup-shaped liquefaction.

Broth. Turbidity and a rather fragile scum appears late. Medium then clears.

Peptone. Turbidity with small patches of surface growth. Medium soon clears.

Potato. Growth abundant, moist, brown with finely wrinkled or lichen-like appearance in the majority of instances. At times the fine wrinkling is lacking and only a thick, moist, brown, mealy growth is produced.

Litmus milk. Slow peptonization with the production of a lilac color turning to amber. In a few weeks digestion is complete and only a white sediment is left behind. No acidification No coagulation.

Blood serum. Thin, white, dry, at times finely wrinkled growth which later becomes yellowish and moist. Suggestion of liquefaction, but this is never complete.

Fermentation tubes. Glucose. Turbidity and scum in bulb and turbidity in closed arm. Reaction acid.

Saccharose. Turbidity in open bulb and usually no scum. Turbidity in closed arm. Reaction acid.

Lactose. Turbidity in open bulb. No scum. Arm clear. Reaction alkaline.

Thermal death point. Spores survive one hour's heating in Arnold sterilizer and autoclaving at 19 pounds pressure. Killed by 20 pounds pressure.

Bacillus globigii Migula.

This organism was originally described by Globig (1888) as *Bacillus mesentericus-ruber.* A culture was obtained from Kral's Laboratory in Vienna which has the same cultural reactions as those given by Globig.

Morphology. Homogeneous bacilli measuring 0.5 by 2 to 3 microns in 24 hours agar cultures. On glucose agar the organisms are longer and slightly thicker often growing out into long chains but short forms are also frequently seen (Figure 14).

Spore formation. Spores are formed very sparsely and at a late period in the present culture. They are usually seen only in 16 to 18 days growth and are then characteristic mesentericus spores.

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-negative.

Agar slant. Thin, spreading, glassy, soft, yellowish-white growth along line of inoculation.

Agar stab. Slight uniform growth along line of puncture with spreading amoeboid surface growth.

Agar colonies. Dense, soft, white amoeboid colonies similar to those of *Bacillus mesentericus.*

Litmus glucose agar slant. Thick, narrow, white growth along line of inoculation. The medium shows an acid reaction.

Litmus glucose agar colonies. Thick, round, raised, soft colonies later turning yellowish and rarely pinkish. Medium acidified.

Gelatin stab. Growth along line of inoculation and slight surface growth with liquefaction of the gelatin.

Gelatin colonies. Surface colonies round, granular, punctiform with slow liquefaction. Some of the larger colonies are spreading and have a glassy surface. Deep colonies punctiform, spherical with dense centers.

Broth. Slight turbidity with no surface growth but a flocculent precipitate. Medium is eventually turned dark yellow.

Peptone. Slight turbidity with no surface growth and no precipitate.

Potato. Yellow, moist growth becoming a reddish brown. Medium is discolored.

Milk. No change in 48 hours. In twenty days the medium shows an acid reaction with the precipitation of a white sediment.

Blood serum. Thick, transparent spreading growth with irregular edges.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Reaction acid.

Saccharose. Turbidity. No scum. No growth in closed arm. Reaction alkaline.

Lactose. Turbidity. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood one hour's sterilizing in the Arnold sterilizer, survived autoclaving at 15 pounds pressure but were killed by 16 pounds pressure.

Bacillus aterrimus Lehmann & Neumann.

This organism was originally described by Biel (1896) and named by Lunt (1896) *Bacillus mesentericus-niger.* It is not uncommon in milk, soil, and the intestinal contents of man.

Morphology. Bacilli similar to *Bacillus vulgatus* in morphology. On plain agar they are homogeneous with blunt ends and measure about 0.5 by 2 to 3 microns in dimensions. On glucose agar they are thicker and longer measuring 0.75 by 2 to 4 microns but at the same time shorter forms are frequent measuring 0.75 by 1.5 microns. (Figures 10 and 11.)

Spore formation. Spores are formed early appearing in 24 hours on plain agar. They form in the center or towards one

end of the rods which do not swell appreciably. When free they may retain spurs of protoplasm at each end unequal in quantity and measure about 0.5 by 1.5 microns. The spores rapidly lose their rims of protoplasm and are then oval to cylindrical measuring 0.5 by 0.75 microns.

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Cultural reactions. This organism is identical with *Bacillus vulgatus* in all its cultural reactions except that it imparts a distinct color to the various media. This color varies from a steel grey to a brown or black and is best seen on solid media. It is very pronounced on potato where the characteristic folds of the "*vulgatus*" are converted to thick black wrinkling bands.

Thermal death point. The spores resist an hour's steaming in the Arnold sterilizer and 15 pounds pressure in the autoclave. They are destroyed by 16 pounds pressure.

Bacillus niger Migula 1900

This organism was first described by Gorini (1894) in 1894 as *Bacillus lactis-niger* and is closely related to the preceding organism. A culture obtained from Kral's Laboratory shows the following reactions.

Morphology. Bacilli with homogeneous protoplasm and blunt or rounded ends measuring 0.375 to 0.75 by 1.5 to 3 microns in 24 hour agar cultures. No change in morphology on glucose agar. (Figures 12 and 13.)

Spore formation. Spores are formed in 24 hours on plain agar and in 48 hours on glucose agar. They appear in the center or towards one end of the rods and are oval or cylindrical in shape. The free spores may retain protoplasm at both ends and are typical of the "mesentericus" group. They measure 0.75 to 1 by 1.125 to 1.25 microns in dimensions.

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Cultural reactions. This species has the general cultural reactions of *Bacillus mesentericus.* It grows on agar as a rather

thick moist mass with a silvery sheen which shows black areas at the edges and in old cultures imparts a black tone to the agar. It liquefies gelatin rapidly, produces a faint acidity in milk which it first coagulates and then slowly digests. On glucose agar it tends to wrinkle slightly. It produces a faint acid in glucose, saccharose and lactose fermentation tubes. On potato it grows as a raised brown mass and it also produces a brownish growth on blood serum.

Thermal death point. The spores withstand boiling one hour in the Arnold sterilizer and a pressure of 20 pounds in the autoclave. They are destroyed by a pressure of 22 pounds.

Bacillus pumilus Gottheil 1901

An organism described by Gottheil (1901) in 1901 as *Bacillus pumilus* is regarded by Chester as identical with *Bacillus mesentericus*. A culture of *Bacillus pumilus* received from Kral's collection in Vienna has all the morphological, tinctorial, developmental and cultural reactions of this species.

Bacillus mycoides Flügge 1886

This organism was first described by Flügge (1886) in 1886 and has since then been given other names by various authors. It is not the same as *Bacillus ramosus-liquefaciens* of Prausnitz which is a distinct species. *Bacillus mycoides* is quite common in Baltimore and is present in milk, water, soil, and dust.

Morphology. In young cultures 6 to 8 hours old on plain agar the organisms are homogeneous with square ends and measure usually a little more than 0.5 micron in width by 3 to 6 microns in length. They are distinctly thinner and longer than *Bacillus cereus*. As the organisms mature the protoplasm appears more granular and a characteristic arrangement in short and long chains is seen. They then resemble the anthrax bacillus. On glucose agar the bacilli are thicker, 0.75 to 1 micron, and usually about the same length. On this medium the protoplasm is converted into globular bodies which do not take the stain and which are similar to those seen in *Bacillus cereus*. In certain

instances the organisms seem to be made up of a network of fine strands in which the globular bodies hang suspended. Often the chains are curled or curved upon themselves. Old cultures show an abundance of swollen involution forms, which seem to have a skein-like arrangement. (Figures 22, 23 and 24.)

Motility. Active motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores begin to form early, appearing first as small refractile bodies in the centers or towards one end of the organisms usually at the end of 24 to 48 hours. Gradually the organisms swell and the spores at the same time increase in size and at this stage a long chain of organisms each containing a spore may often be seen. The protoplasm soon disintegrates leaving a rim about the spore which is round or oval or slightly rectangular. Such spores measure 0.75 to 1 by 1.125 microns. Other spores are more definitely elongated and may measure 0.75 to 1 by 1 to 2 microns. The spores often remain attached to each other in short or long chains. The spores vary more in size than do others of this group and may show small forms 0.375 to 0.5 by 0.5 to 1 and large forms measuring 1.125 by 2 lying side by side.

Slant agar. Filamentous rhizoid growth spreading from the line of inoculation and extending into the agar. This growth is at first glassy and glistening, but later grows dull and soft. Appearance on agar characteristic.

Agar stab. Faint arborescent growth along line of inoculation with a surface development in concentric zones.

Agar colonies. Surface colonies spread from dark dense nuclei and show dense, rhizoid peripheries extending into the agar on all sides. Under low power the periphery of the colony is found to be composed of parallel strands of growth. Deep colonies have almost the same appearance and always exhibit the spreading peripheral myceleoid outgrowths.

Litmus glucose agar. Thin membranous myceleoid growth later becoming branched and reticulate. Growth at first moist and white, later becoming pale yellow. Medium first acidified and then turned deep blue.

Litmus glucose agar colonies. Surface colonies thin, round or irregular. Under low power found to consist of masses of matted filaments with usually dense central nuclei, from which single or parallel strands extend into the agar in every direction for long distances. Deep colonies exhibit the same small, punctiform and matted myceleoid growth, under lower power. Medium first acidified and then made alkaline.

Gelatin stab. Filamentous growth along line of inoculation with surface liquefaction.

Gelatin colonies. Colonies consist of dense central nuclei with matted edges from which long strands emerge. The colonies present a peculiar appearance like a chestnut burr.

Broth. No turbidity but a firm scum forms which is soon precipitated.

Peptone. No turbidity, but a flocculent suspension and a firm scum which is soon precipitated.

Potato. Mealy white, later becoming brownish.

Litmus milk. Slow peptonization to an amber-colored fluid. No acidification. No coagulation.

Blood serum. Dry, myceleoid interlacing luxuriant growth. No liquefaction.

Fermentation tubes. Glucose. Flocculent growth in bowl and arm. Scum forms and is soon precipitated. Reaction acid.

Saccharose. Flocculent in bowl and in arm. Scum is formed and precipitated. Some cultures produce moderate acidity. Others produce no acid.

Lactose. Growth in open bulb with a slight extension into arm. Scum formed and soon precipitated. Reaction alkaline.

Thermal death point. Spores survived one hour in the Arnold sterilizer and 15 pounds pressure in the autoclave. Destroyed by 16 pounds pressure.

Bacillus megatherium De Bary

This organism was originally found and named by De Bary (1884, 1887) and has since beeen described under a variety of names by a number of authors. It is one of the most common of

the spore-bearing bacteria and has been found in dust, soil, milk, water, and as a laboratory contamination. The present description applies to cultures obtained from the Kral collection, and from the American Museum, and to over a hundred of our own isolations.

Morphology. These are the largest of the spore-bearing organisms. On plain agar in young cultures, from 8 to 24 hours old, they are long and thick with homogeneous or slightly granular protoplasm, measuring 0.75 to 1.25 by 3 to 9 microns. On glucose agar they are even thicker measuring 1.25 to 1.5 in width. On both media long forms occur but especially on glucose agar. These may measure 30 to 45 microns in length and may show homogeneous protoplasm without evident segmentation. The protoplasm of the organism is at first homogeneous, but by the end of 24 to 48 hours it is converted into a mass of globular bodies resistant to the stains. These globular bodies are clear, highly refractile, bulge the organism somewhat, and are quite numerous six to eight appearing in each rod. They thus give the organism a peculiar and characteristic appearance. They show most markedly on glucose agar but are also present on plain agar where they can best be demonstrated by decolorizing an over-stained preparation. Their nature is not clear as they do not take any special bacterial stains. Shadow or transparent forms appear in *Bacillus megatherium* early, both on plain and glucose agar. These measure 1.125 to 1.5 by 4 to 10 microns, take the stain very faintly and show peculiar bodies of agglomerated protoplasm at the sides or sometimes at the ends. These transparent forms are often thicker and longer and may even measure 2 by 40 to 45 microns. Occasionally they are distinctly oval with rounded ends measuring about 1.5 by 4 microns and show a small bunch of cytoplasm at the side. When these forms are in chains they are exactly like the original pictures of De Bary. (Figures 31, 32, 33, 34, 35, 36, 37).

Motility. Active progressive and rotatory motility in young cultures.

Staining properties. Gram-positive.

Spore formation. Spores are formed abundantly on plain

agar in 24 hours and on glucose agar in 48 hours. They appear in the center or slightly towards one end of the rods and are usually of the same diameter but may be slightly thicker. Sometimes two spores seem to arise in one rod but these may possibly be in a rod just prior to division. In general each rod has a single spore. The spores occasionally lie obliquely in the rods. Frequently two spores are at opposite ends of rods lying in juxtaposition and these may remain attached in chains and present a characteristic appearance. The free spores retain protoplasm at the ends for some time. When this is unequal in amount the spore has somewhat the shape of a tennis racket and handle. The free spores are oval to cylindrical and measure 0.75 to 1.125 by 1.5 to 2 microns. They are often flattened on one side having an appearance described as kidney shaped or reniform. The spores show great variations in size more so than do those of the other members of this group.

Slant agar. Thick, raised, soft, white or cream-colored growth which shows a pink tinge by reflected light, with many small, minute pellucid areas. As the cultures get older the growth becomes pale yellow.

Agar stab. Slight growth along line of inoculation, heaped up and spreading slightly on surface. Later surface growth becomes slightly pinkish.

Agar colonies. Surface colonies round, thick, white or cream-colored, highly refractive, turning pale yellow or yellowish-brown in old cultures. Under low power slightly granular, brownish yellow, with entire margins. Deep colonies punctiform. Under low power round or irregular with entire edges, brown and granular.

Litmus glucose agar slant. Thick, luxuriant growth along line of inoculation, at first white and then pale yellow or cream-colored. Medium is first acidified but later becomes alkaline and changes from a dark blue to a smoky brown while the growth becomes a dark gray or smoky brown.

Litmus glucose agar colonies. Large, round, raised surface colonies, cream colored to pale yellow, with heaped up central nuclei. Under low power dark, slightly granular with entire

edges. Deep colonies punctiform. Under low power dark, irregular, bizarre, with entire edges. Medium is acidified and then made alkaline.

Gelatin stab. Funnel-shaped liquefaction. No scum.

Gelatin colonies. Round colonies with concentric zones of growth. Under low power cloudy central nuclei with filamentous peripheries.

Broth. Turbidity but no scum.

Peptone. Turbidity but no scum.

Potato. Thick, white, mealy growth later becoming pale or cream yellow.

Litmus milk. No change within 24 hours then a gradual peptonization with the production of a port-wine colored fluid. No acidification. No coagulation.

Blood serum. Thick, white, moist, heavy growth cream white to yellow in color. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Saccharose. Turbidity in open bulb. No scum. No growth in closed arm. Acid production feeble.

Lactose. Slight turbidity in open bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores withstood 1 hour steaming in the Arnold sterilizer and 18 pounds pressure in the autoclave. Killed by 19 pounds pressure.

Bacillus petasites Gottheil 1901

This organism was described originally by Gottheil (1901) in 1901. Its chief point of differentiation from *Bacillus megatherium* is that it produces a distinct yellow pigment on artificial media, particularly on plain agar and on potato. It is extremely common, having been found in dust, soil, water, milk, and various milk products. The present description applies to a culture from the Kral collection and to over a hundred of our own isolations.

Morphology. The organisms do not differ appreciably in morphology from *Bacillus megatherium.* They are homogeneous

or slightly granular rods measuring 0.75 to 1.5 by 3 to 6 microns on plain agar in young cultures (8 to 24 hours), and 1 to 1.75 by 3 to 6 on glucose agar. Long forms measuring 12 to 25 microns are seen on plain and on glucose agar. Shadow forms with faintly staining protoplasm, like those seen in *Bacillus megatherium* are common as well as the peculiar refractile globular bodies. (Figures 38, 39, 40, 41 and 42.)

Motility. Active progressive and rotatory motility in young cultures.

Staining reactions. Gram-positive.

Spore formation. Spores are formed abundantly in 24 hours on plain and on glucose agar. The spores are oval to rectangular, of about the same width as the rods from which they spring and frequently form long chains. The free spores may retain tags of protoplasm but soon lose them and then show great variations in size and shape. They may be nearly round, oval, rectangular and reniform and measure 0.75 to 1 by 1.5 to 2 microns.

Agar slant. Thick, moist, abundant mealy growth at first slightly pinkish by reflected light, then becoming bright lemon yellow. Agar slightly discolored.

Agar stab. Slight growth along line of inoculation with heaped up yellowish growth on surface.

Agar colonies. Surface colonies thick, white or yellow, highly refractive. Under low power dark, granular with entire or myceleoid edges. Deep colonies punctiform. Under low power irregular, with irregular edges showing myceleoid, rooty or fuzzy edges.

Litmus glucose agar. Luxuriant, thick, heaped-up growth at first yellow then assuming an orange and then a dark-brown color. Reaction of medium first acid then alkaline. It eventually becomes smoky-brown.

Litmus glucose agar colonies. Surface colonies round, regular and thick or thin and spreading. Under low power granular with entire edges. Deep colonies punctiform. Under low power granular, irregular, with fuzzy edges. Reaction of medium acid at first then alkaline.

Gelatin stab. Growth along line of inoculation with funnel-shaped surface liquefaction. No scum formation.

Broth. Turbidity. No scum. Medium eventually becomes yellow.

Peptone. Turbidity. No scum.

Potato. Thick, mealy, bright yellow growth gradually becoming dark yellow.

Litmus milk. No change in 24 hours then a gradual peptonization with the production of a port-wine-colored fluid.

Blood serum. Thick, dry, yellowish, moist growth becoming pale to bright yellow. No liquefaction.

Fermentation tubes. Glucose. Slight turbidity in bulb. No scum. No growth in closed arm. Feeble acid production.

Saccharose. Slight turbidity in bulb. No scum. No growth in closed arm. Faint acid production.

Lactose. Slight turbidity in bulb. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. Spores survived steaming in Arnold sterilizer 30 minutes, but were killed by 1 hour exposure. Withstood 19 pounds pressure in autoclave but were killed by 20 pounds pressure.

Bacillus tumescens Zopf 1885

This organism was described by Zopf in 1885 (Zopf, 1885). A culture received from the Kral collection and another received from the American Museum agree in their morphological, developmental, tinctorial and cultural features all of which are identical with those of *Bacillus megatherium.* (Figures 45, 46 and 47.)

Bacillus graveolens Gottheil 1901

This organism was described in 1901 by Gottheil (1901) as a new species. A culture from the Kral collection in Vienna has all the cultural reactions of *Bacillus megatherium.* Morphologically it is about the same size, forms spores in the same way, is Gram-positive, produces globular bodies on plain and glucose agar and undergoes involution with the formation of shadow or

washed-out forms. It shows however a distinct tendency to produce curved or spiral forms. On the basis of this one characteristic it is hardly justifiable to make it a distinct species. It should be noted that this use of the term "*graveolens*" is probably incorrect since a *Bacterium graveolens* was described by Bordoni Uffreduzzi (1886) in 1886. This was a small non-sporulating bacillus producing a green pigment. (Figures 43 and 44.)

Bacillus cohaerens Gottheil 1901.

This organism was described by Gottheil (1901) in 1901 but according to Chester it is identical with *Bacillus simplex*. The culture of *Bacillus cohaerens* received from the Kral collection is distinct from *Bacillus simplex* and is represented by four organisms isolated in Baltimore, one from milk and three from soil. The present description applies to all five strains.

Morphology. Small, rather uniform homogeneous organisms with rounded ends, measuring 0.375 to 0.5625 by 0.75 to 2.25 microns in 24 hour cultures on plain agar. On glucose agar the bacilli are thicker and longer measuring 0.5625 to 0.75 by 2 to 5 microns. On both media shadow forms appear early often in 24 hours. These are made up of faintly-staining protoplasm with deeply-staining particles in various positions, at the ends, towards the center, or at the periphery. (Figures 15, 16, and 17.)

Motility. Actively motile in 24 hour cultures.

Staining properties. Gram-positive.

Spore formation. Spores were formed slowly and sparsely in the Kral culture and in one of ours. They appeared in about 10 days, were oval or elliptical, arose in the centers of the rods which were slightly bulged on sporulation. The free spores were very delicate and stained with difficulty. They measured about 0.5625 by 0.75 microns. In a more recent isolation of our own the spores appeared in 48 hours, were central or excentric, bulged the rods and later retained distinct rims of protoplasm, measuring 0.75 by 1.5 to 1.5 microns. Later the spores lost their protoplasm, became more oval and measured 0.5 to 0.5625 by 0.9375 to 1.25 microns. Rarely the spores retained unequal

bits of protoplasm at the ends and then they resembled the mesentericus spores slightly.

Agar slant. Thin, soft spreading, whitish growth later becoming yellow. Easily scraped off the agar.

Agar stab. Faint growth along line of inoculation and spreading on the surface, thick and whitish in old cultures.

Agar colonies. Surface colonies round or bizarre, thick, white. Under low power granular with dense central nuclei. Edges entire. Deep colonies punctiform. Under low power irrregular, with entire edges.

Litmus glucose agar. Thick, soft, whitish growth along line of inoculation becoming yellowish and irregularly heaped up. Medium quite markedly acidified.

Litmus glucose agar colonies. Surface colonies round or irregular, thick, whitish. Under low power granular and frequently show dense central nuclei with thin peripheries showing regular edges. Deep colonies punctiform. Under low power irregular with irregular edges. Reaction of medium acid.

Gelatin stab. Faint growth along line of inoculation with surface liquefaction and scum production.

Gelatin colonies. Thin, circular colonies, under low power granular.

Broth. Turbidity at first, then the medium clears and a dense surface growth appears which shows many clear, globular masses like globules of fat floating on the surface.

Peptone. Turbidity with a faint fragile scum.

Potato. Thin, spreading, moist, yellow growth.

Litmus milk. Slow decolorization of the litmus with peptonization and the production of an amber-colored fluid.

Blood serum. Thin, whitish growth. No liquefaction. May appear finely wrinkled.

Fermentation tubes. Glucose. Turbidity in bowl with surface growth and flocculent precipitate. Arm clear. Reaction acid at the end of 2 to 3 days.

Saccharose. Turbidity in bowl with slight surface growth. Arm clear. Acidity at the end of 2 to 3 days.

Lactose. Turbidity in bowl with very slight surface growth. Arm clear. Reaction alkaline.

Thermal death point. In one isolation the spores survived one hour steaming in the Arnold sterilizer; and withstood 18 pounds pressure in the autoclave but were killed by 19 pounds pressure. In another isolation from soil the spores survived 14 pounds pressure in the autoclave but were killed by 16 pounds. They survived one hour steaming in the Arnold.

Bacillus simplex Gottheil 1901

This organism was described by Gottheil (1901) in 1901 as a distinct species. According to Chester it is the same as *Bacillus cohaerens* of Gottheil. Cultures of both organisms have been received from Kral's Laboratory in Vienna and can easily be differentiated. The present description applies to the Kral culture and to an organism obtained from soil by Dr. Laubach. The species is evidently one of the rare spore-bearing organisms.

Morphology. In the Kral culture the organisms are large homogeneous rods with rounded ends, measuring usually 0.5625 to 0.75 by 3 to 4.5 microns. At times much thicker forms are seen approximating 1.125 micron in thickness while longer forms 6 microns in length are not uncommon. The organisms often grow out into long threads or filaments 10 to 12 microns in length, especially on glucose agar. Even in young cultures the homogeneous rods lose their protoplasm and are converted into peculiar shadow forms. These are made up of a very faintly staining protoplasm in which denser aggregations of cytoplasm appear. Such forms measure 1.125 to 1.25 by 12 to 15 microns in dimensions. On glucose agar the organisms have the same morphology but may show an abundance of shadow forms. Involution and shadow forms are very abundant in old cultures. In our own isolation the organisms, while somewhat smaller, did not differ appreciably in morphology, measuring 0.5 to 0.5625 by 1.5 to 2.5 microns but also showing both the thicker and longer forms seen in the Kral culture and the characteristic shadow and involution forms. Long forms were also very common on glucose agar. (Figures 18, 19, 20, and 21.)

Motility. Actively motile in young cultures.

Staining properties. Gram-positive.

Spore formation. In the Kral culture the spores were at first formed very slowly appearing only after the lapse of 15 to 16 days. Subsequently after repeated transfers, spore formation became more active and spores were often formed in 24 hours. They appeared in the centers or towards one end of the rods, were no thicker than the rods from which they sprung, and were cylindrical or almost rectangular in shape. They retained rather thick walls of protoplasm for some time and measured 0.5625 by 1.125 to 1.25 microns. In our own isolation the spores were formed in 48 to 72 hours in the same way as in the Kral culture but were a trifle smaller measuring 1.375 to 0.5 by 0.75 to 1 micron.

Agar slant. Thin, translucent, slightly yellowish gelatinous growth, gradually becoming denser and developing occasionally a dry slightly wrinkled surface. Single accessory colonies not uncommon at the edges of the main growth.

Agar stab. Slight uniform growth along line of puncture with a thick circular surface growth.

Agar colonies. Surface colonies thin, translucent, amoeboid developing from pin-point centers. Under low power granular. Deep colonies round or oval, regular, granular, with clean or rarely irregularly fuzzy edges.

Litmus glucose agar. Thick, abundant yellowish-white, heaped up growth with serrated margins. Medium faintly acidified in old cultures.

Litmus glucose agar colonies. Superficial colonies thin, smooth, white and soft. Under low power granular, edges irregular but entire. Deep colonies punctiform. Under low power irregular with irregular rarely fuzzy margins. A trace of acid usually produced.

Gelatin stab. Faint growth along line of inoculation with cup-shaped surface liquefaction.

Gelatin colonies. Round, thick, whitish colonies with concentric rings and sharply defined edges. Medium liquefied.

Broth Faint turbidity, slight sediment, no scum but rarely a faint ring growth along side of tube.

Litmus milk. Gradual clearing with production of straw-colored fluid in the Kral culture. In our own isolation a gradual

clearing to a port-wine fluid. No coagulation. Later straw-colored.

Peptone. Faint turbidity and sediment with rarely a slight ring growth.

Potato. Thick, moist, abundant, gelatinous, yellowish-brown growth.

Blood serum. Thin, spreading, whitish growth. No liquefaction.

Fermentation tubes. Glucose. Turbidity in open bulb. No scum, arm clear. Reaction neutral or slightly acid.

Saccharose. Faint turbidity in bulb. No scum. Arm clear. Reaction alkaline.

Lactose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Thermal death point. In the Kral culture the spore survived steaming in the Arnold sterilizer for 15 minutes. They withstood a pressure of 15 pounds in the autoclave but were destroyed by 16 pounds pressure. In our own isolation the spores survived 10 pounds in the autoclave but were killed by 12 pounds pressure. They survived 15 minutes steaming in the Arnold sterilizer but were killed by 30 minutes steaming.

Bacillus fusiformis[2] Gottheil 1901

This organism was first described by Gottheil (1901) in 1901. A transfer from Gottheil's original was obtained from Kral's Laboratory in Vienna. Fourteen organisms corresponding closely to Gottheil's isolation were obtained in Baltimore, two from milk, four from dust, two from water, five from soil and one from contaminated hirudin. The present description applies to all of them.

[2] *Bacillus fusiformis* has practically the same morphology and the same cultural reactions as the organism described in 1909 by Jordan and Harris as the cause of milksickness and named by them *Bacillus lactimorbi* (Journal of Infectious Diseases, Vol. 6, No. 4, September 20, 1909, p. 401). A culture of *Bacillus lactimorbi* received from the Winslow collection in New York does not differ appreciably in its reactions from the strains of *Bacillus fusiformis* in our laboratory. Without a thorough study of pathogenicity, however, it is impossible to state whether the organisms found by us are identical with *Bacillus lactimorbi* or not.

Morphology. Thick stubbed homogeneous organisms with round or pointed ends usually appearing as single cells or in twos. No chain formation. On 24 hour plain agar cultures they measure 0.5 to 0.75 by 1.5 to 2 microns. Organisms not increased in size on glucose agar and protoplasm remains homogeneous. Sometimes long forms 6 to 8 microns appear in old cultures. (Figures 48, 49, 50 and 51.)

Motility. Active progressive and rotatory motility in 24 agar cultures.

Staining properties. Gram-negative.

Spore formation. Spores form early appearing in 24 hours on both plain and glucose agar. They are round, greater in diameter than the organisms from which they spring, and are usually located at the ends of the rods in a terminal or subterminal position. They thus give a clavate or club-shaped appearance to the rods which resemble somewhat the tetanus bacillus. The spores may also be central and the rods thus become fusiform in shape. The free spores may retain spurs of protoplasm assuming a peculiar diamond shape or may appear naked. They vary in diameter from 0.5 to 1 micron and are occasionally swollen equaling 1.5 microns in thickness.

Agar slant. Thick white rather dry growth in 24 hours, becoming distinctly yellow or cream-colored in old cultures. Easily scraped from medium.

Agar stab. Faint line growth and non-spreading surface growth.

Agar colonies. Superficial colonies may be round, regular thick and opaque, or thin and spreading. Under low power they show dark central nuclei and thinner margins with clean-cut edges. Older cultures thick and heaped up. Deep colonies small and fine, under low power dark, opaque, round or irregular.

Glucose agar. Thick dry growth with heaped-up edges becoming thicker and granular in old cultures. Reaction alkaline.

Glucose agar colonies. Superficial colonies thick, irregular spreading and heaped up. Under low power granular with irregular fuzzy margins. Deep colonies opaque under low power showing irregular fuzzy edges. Older colonies thicker and more bizarre-shaped. Reaction alkaline.

Gelatin stab. Growth along line of inoculation with cup-shaped or funnel-shaped liquefaction. Dense turbidity in the liquefied gelatin with a thick scum. Gelatin may be faint pink in color.

Gelatin colonies. Small fine colonies round and regular or irregular and spreading. Under low power they show fine hairy outgrowths. Gelatin slowly liquefied.

Broth. Turbidity and fine sediment. No scum.

Peptone. Turbidity and fine sediment. No scum.

Potato. Faint yellow growth becoming yellowish brown in old cultures.

Litmus milk. Gradual reduction of the litmus and slow but complete digestion of the proteins. No coagulation.

Blood serum. Non-spreading cream yellow growth becoming yellowish brown in old cultures. No liquefaction.

Fermentation tubes. Glucose. Turbidity in bowl. Arm clear. No scum. Reaction alkaline.

Saccharose. Reactions the same.

Lactose. Reactions the same.

Thermal death point. Spores destroyed by steaming 15 minutes in the Arnold sterilizer. They survive $7\frac{1}{2}$ pounds in the autoclave but are destroyed by 10 pounds pressure.

Bacillus terminalis Migula 1900

This organism was first obtained by Flügge (1894) in 1894 and called by him, No. XII. It was subsequently correctly named *Bacillus terminalis* by Migula and still later named *Bacillus lacteus* by Chester (1901). On two occasions we have isolated organisms which have the same morphology and method of spore-formation as *Bacillus terminalis* but differ slightly in cultural reactions. It does not seem wise to make a new species since our strains may represent merely attenuated varieties of Flügge's organism. The following description is taken from our own isolations and the points of differentiation between them and Flügge's original isolation are indicated.

Morphology. Long thin bacilli with slightly granular protoplasm measuring 0.375 by 2.25 to 4 microns in 24 hour agar cul-

tures. On glucose agar the organisms retain the same diameter but grow out into long chains which often assume spiral arrangements. (Figure 52.)

Spore formation. Spores are formed slowly seldom appearing before 48 hours. They are cylindrical, thicker than the rods from which they spring, terminal or sub-terminal, giving the organisms a clavate or club-shaped appearance. Free spores are 0.75 by 1.5 microns in dimensions.

Motility. Active motility in 24 hour cultures.

Staining properties. Gram-negative.

Agar slant. Thin spreading smooth glistening growth with gradual darkening of the agar.

Agar stab. Faint growth along line of puncture and on the surface at the point of inoculation.

Agar colonies. Colonies grow slowly appearing only after 3 to 4 days. They are round, regular, under low power showing central nuclei with thin spreading peripheries. Deep colonies apt to be irregular under low power, showing clean-cut or entire edges.

Glucose agar. Faint white filmy growth with an alkaline reaction.

Glucose agar colonies. Thin slow-growing spreading surface colonies, under low power showing dense central nuclei and thin margins. Deep colonies punctiform, under low power slightly granular with irregular margins. Reaction alkaline.

Gelatin stab. Growth along line of inoculation and slow cup-shaped liquefaction.

Gelatin colonies. Colonies on the surface show dense central nuclei and concentric spreading peripheral margins. Deep colonies punctiform and tend to show same arrangement. Under low power edges entire.

Broth. Slight turbidity. No scum. No sediment. Fragile scum described by Flügge.

Peptone. Slight turbidity. No scum. No sediment.

Potato. No visible growth in our isolations. Faint moist growth gradually becoming thicker and yellowish, noted by Flügge.

Milk. No change produced by our strains. Slow peptonzation described by Flügge.

Blood serum. Thin transparent spreading growth, pale yellow to yellowish-brown. No liquefaction. Slight sinking-in of the growth mentioned by Flügge.

Fermentation tubes. Glucose. Faint turbidity in bowl. No scum. No growth in closed arm. Reaction alkaline.

Saccharose. Appearance the same. Reaction alkaline.

Lactose. Appearance the same. Reaction alkaline.

Thermal death point. Spores survived 10 pounds in autoclave but were killed by 15 pounds pressure.

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ILLUSTRATIONS

The illustrations were drawn by Mrs. W. W. Ford and Mr. W. P. Didusch from preparations illustrating the different phases in the morphology of the various species. The preparations were always stained by gentian violet and drawn from a Zeiss microscope with a 1/12 oil immersion lens and a compensating ocular No. 8, giving a magnification of 1080 diameters. The attempt was made to show the morphology of the vegetative cells which comes out best in certain species at 6 to 7 hours and in others at 22 to 24 hours, the method of spore-formation which varies in the time in which it appears, and the changes which occur in the organism when grown on carbohydrate media.

EXPLANATION OF PLATES

PLATE 1

FIG. 1. *Bacillus coli.* Plain agar, 24 hours
FIG. 2. *Bacterium anthracis.* Plain agar, 24 hours

PLATE 2

FIG. 3. *Bacterium anthracis.* Plain agar, 4 days
FIG. 4. *Bacillus subtilis* from milk. Plain agar, 20 hours

PLATE 3

FIG. 5. *Bacillus subtilis* from milk. Glucose agar, 24 hours
FIG. 6. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*). Plain agar, 20 hours

PLATE 4

FIG. 7. *Bacillus vulgatus* from milk (*Bacillus mesentericus vulgatus*). Glucose agar, 24 hours
FIG. 8. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*). Plain agar, 72 hours

PLATE 5

FIG. 9. *Bacillus mesentericus* from soil (*Bacillus mesentericus fuscus*). Glucose agar, 48 hours
FIG. 10. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*). Plain agar, 20 hours

PLATE 6

FIG. 11. *Bacillus aterrimus* from human intestinal contents (*Bacillus mesentericus niger*). Glucose agar, 48 hours
FIG. 12. *Bacillus niger* from Kral (*Bacillus lactis niger*). Plain agar, 48 hours

PLATE 7

FIG. 13. *Bacillus niger* from Kral (*Bacillus lactis niger*). Glucose agar, 48 hours

FIG. 14. *Bacillus globigii* from Kral (*Bacillus mesentericus ruber*). Plain agar, 20 hours

PLATE 8

FIG. 15. *Bacillus cohaerens* from milk. Plain agar, 7 hours

FIG. 16. *Bacillus cohaerens* from soil. Plain agar, 6 hours

PLATE 9

FIG. 17. *Bacillus cohaerens* from soil. Plain agar, 24 hours

FIG. 18. *Bacillus simplex* from Kral. Plain agar, 5 hours

PLATE 10

FIG. 19. *Bacillus simplex* from Kral. Plain agar, 20 hours

FIG. 20. *Bacillus simplex* from soil. Plain agar, 24 hours

PLATE 11

FIG. 21. *Bacillus simplex* from soil. Plain agar, 3 days

FIG. 22. *Bacillus mycoides* from cow dung. Plain agar, 5 hours

PLATE 12

FIG. 23. *Bacillus mycoides* from cow dung. Plain agar, 24 hours

FIG. 24. *Bacillus mycoides* from cow dung. Plain agar, 5 days

PLATE 13

FIG. 25. *Bacillus cereus* from milk. Plain agar, 7 hours

FIG. 26. *Bacillus cereus* from milk. Plain agar, 24 hours

PLATE 14

FIG. 27. *Bacillus cereus* from milk. Glucose agar, 24 hours

FIG. 28. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Plain agar, 7 hours

PLATE 15

FIG. 29. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Plain agar plate, 24 hours

FIG. 30. *Bacillus albolactus* from milk (*Bacillus lactis albus*). Glucose agar, 24 hours

PLATE 16

FIG. 31. *Bacillus megatherium* from American Museum. Plain agar, 7 hours

FIG. 32. *Bacillus megatherium* from American Museum. Plain agar (plate), 24 hours

PLATE 17

FIG. 33. *Bacillus megatherium* from American Museum. Glucose agar, 24 hours

FIG. 34. *Bacillus megatherium* from American Museum. Glucose agar, 48 hours

PLATE 18

FIG. 35. *Bacillus megatherium* from Kral. Plain agar, 7 hours

FIG. 36. *Bacillus megatherium* from Kral. Plain agar, 24 hours

PLATE 19

FIG. 37. *Bacillus megatherium* from Kral. Glucose agar, 20 hours

FIG. 38. *Bacillus petasites* from milk. Plain agar, 7 hours

PLATE 20

FIG. 39. *Bacillus petasites* from milk. Plain agar, 20 hours

FIG. 40. *Bacillus petasites* from milk. Plain agar, 48 hours

PLATE 21

FIG. 41. *Bacillus petasites* from Kral. Plain agar, 7 hours

FIG. 42. *Bacillus petasites* from Kral. Plain agar, 20 hours

PLATE 22

FIG. 43. *Bacillus graveolens* from Kral. Plain agar, 7 hours

FIG. 44. *Bacillus graveolens* from Kral. Plain agar, 20 hours

PLATE 23

FIG. 45. *Bacillus tumescens* from Kral. Plain agar, 7 hours

FIG. 46. *Bacillus tumescens* from Kral. Plain agar 20 hours

PLATE 24

FIG. 47. *Bacillus tumescens* from Kral. Glucose agar, 48 hours

FIG. 48. *Bacillus fusiformis* from dust. Plain agar, 24 hours

PLATE 25

FIG. 49. *Bacillus fusiformis* from dust. Plain agar, 48 hours, showing long threads

FIG. 50. *Bacillus fusiformis* from contaminated hirudin. Plain agar, 24 hours

PLATE 26

FIG. 51. *Bacillus fusiformis* from contaminated hirudin. Plain agar, 48 hours

FIG. 52. *Bacillus terminalis* from milk. Plain agar, 17 days

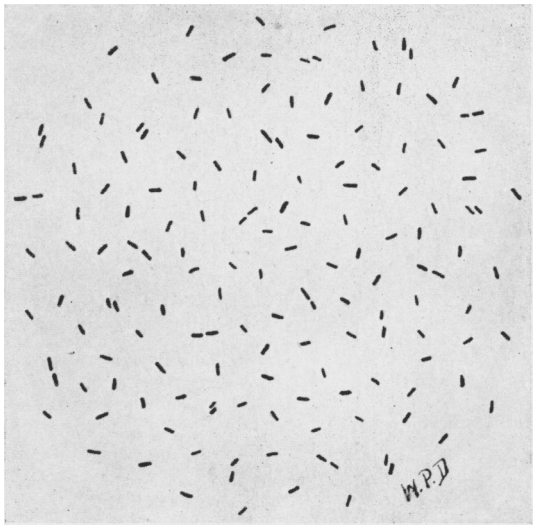

FIG. 1

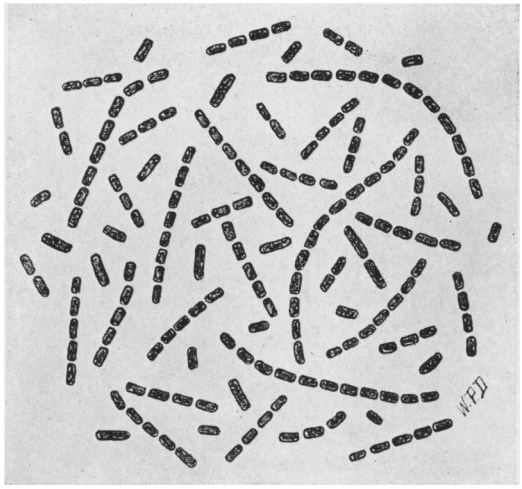

FIG. 2

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

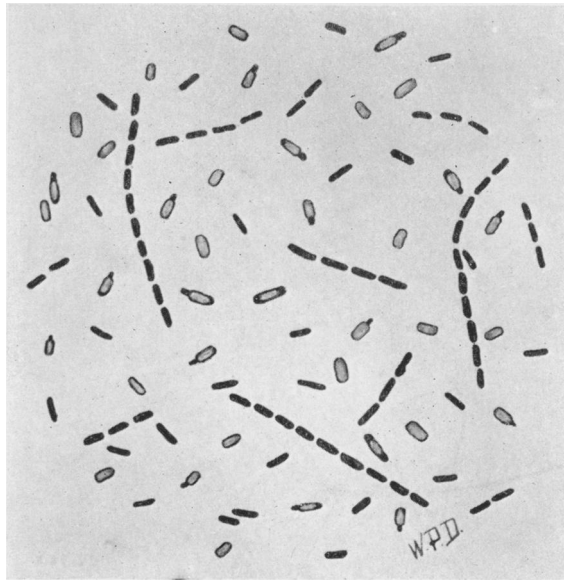

FIG. 3

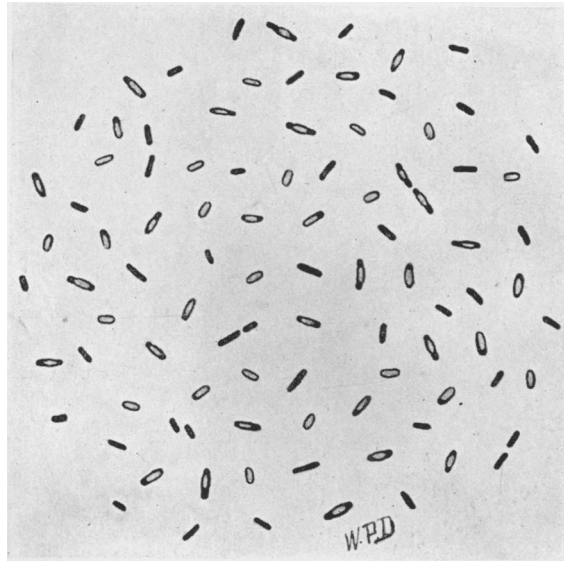

FIG. 4

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

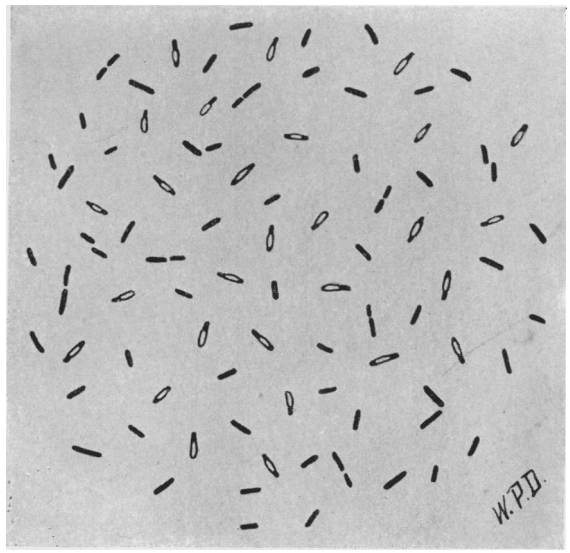

FIG. 5

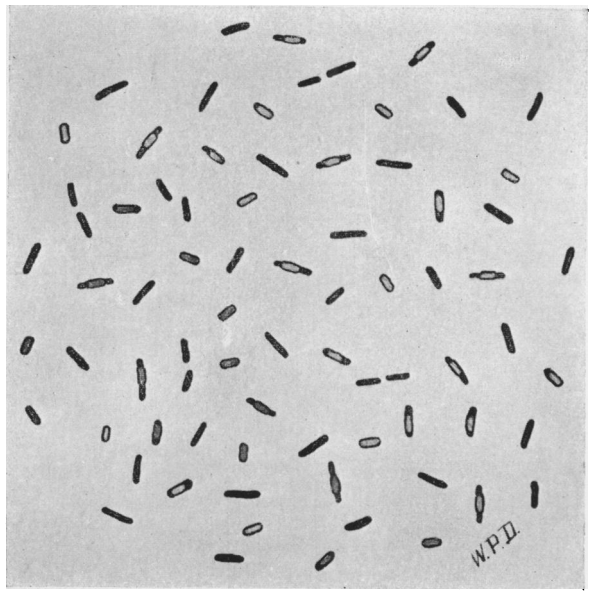

FIG. 6

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

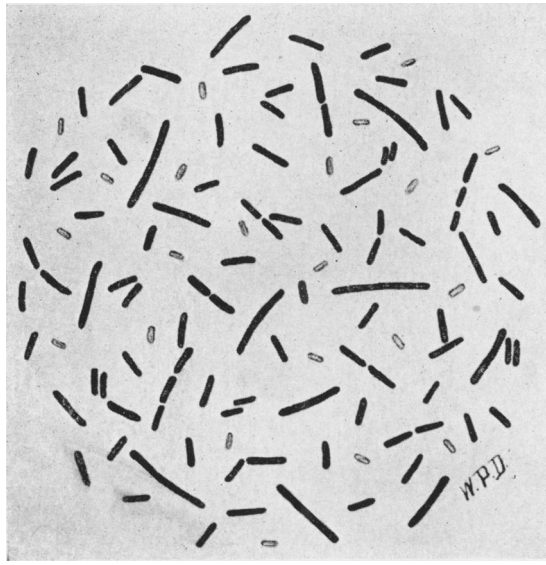

FIG. 7

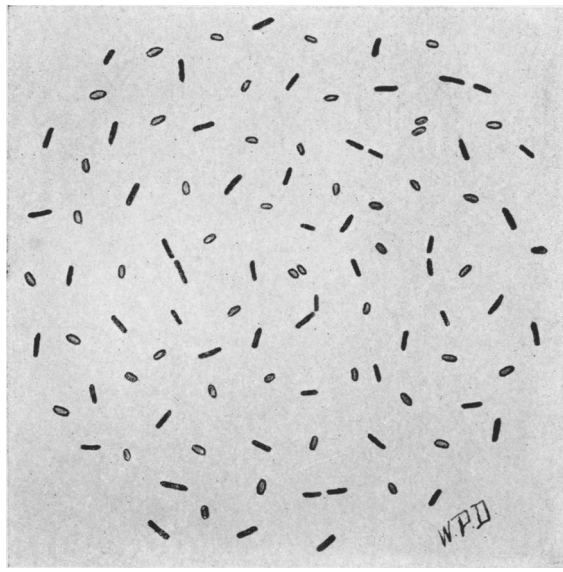

FIG. 8

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

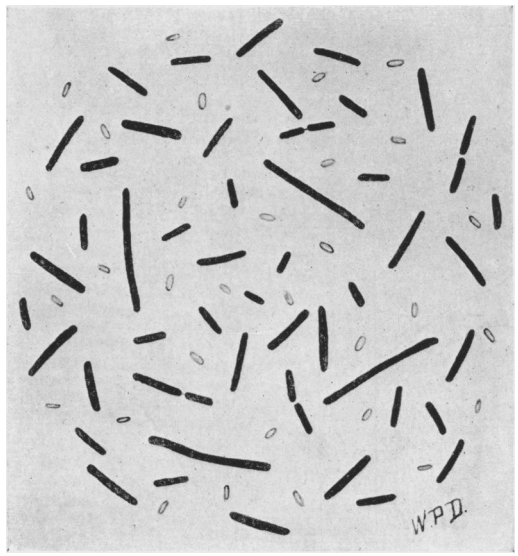

FIG. 9

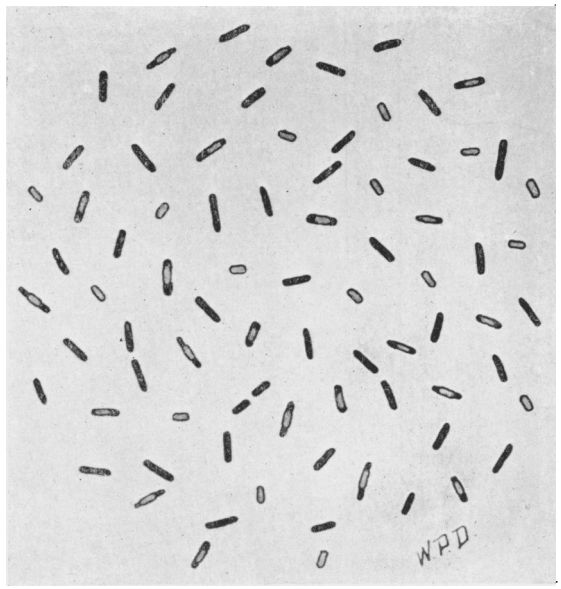

FIG. 10
(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

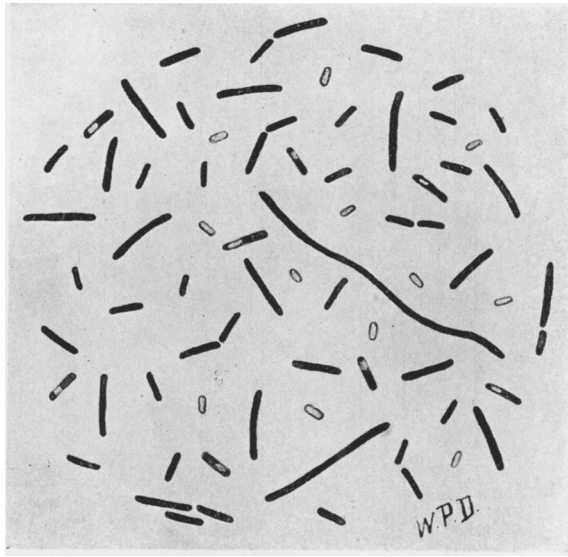

FIG. 11

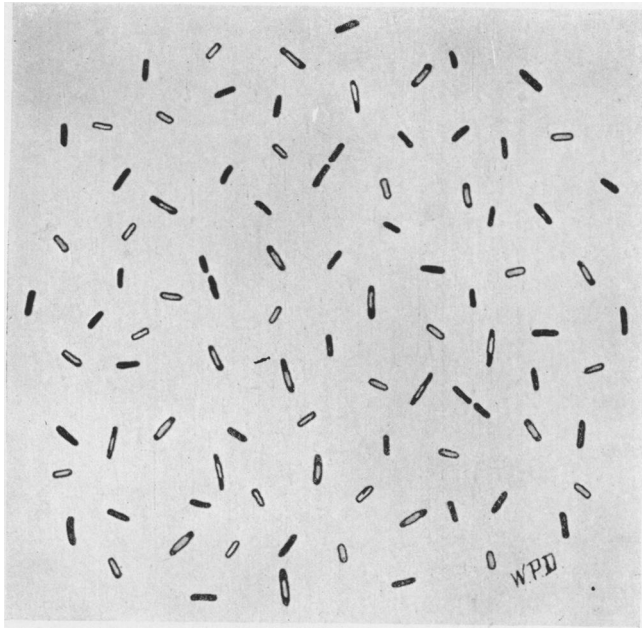

FIG. 12

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

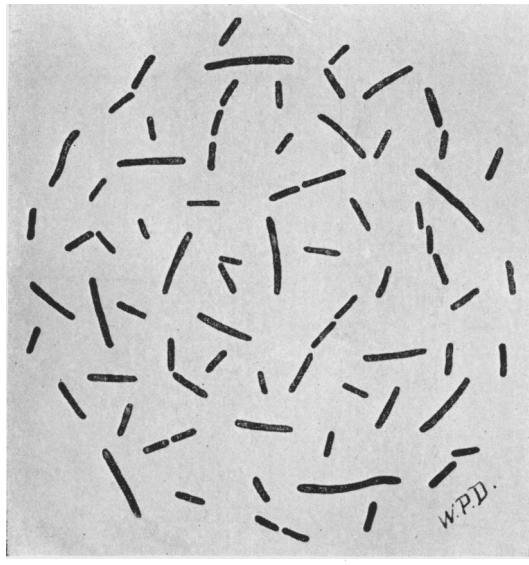

FIG. 13

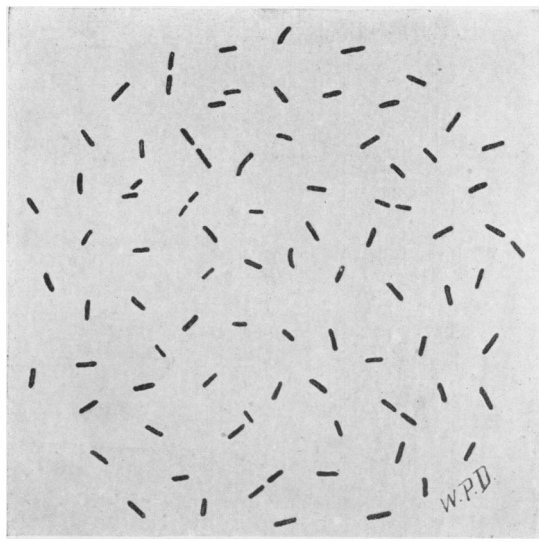

FIG. 14

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

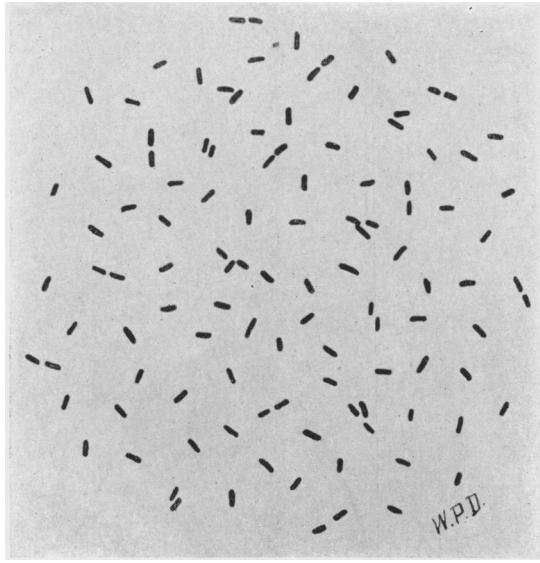

FIG. 15

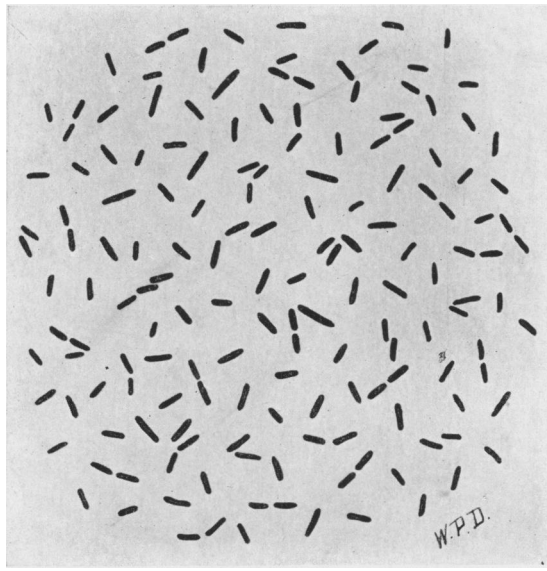

FIG. 16

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

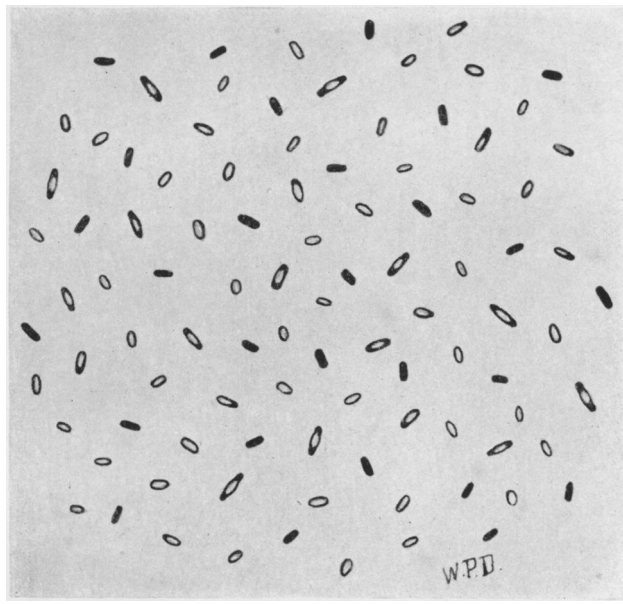

FIG. 17

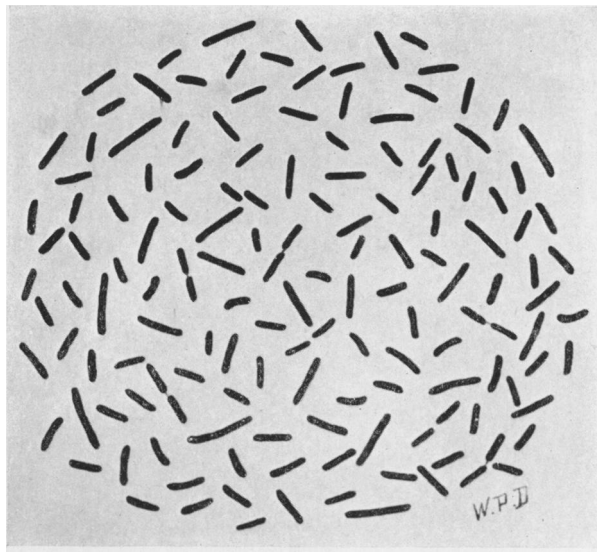

FIG. 18

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

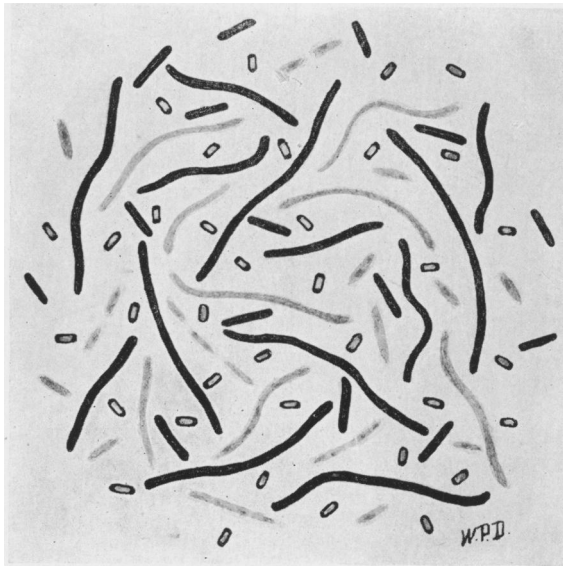

FIG. 19

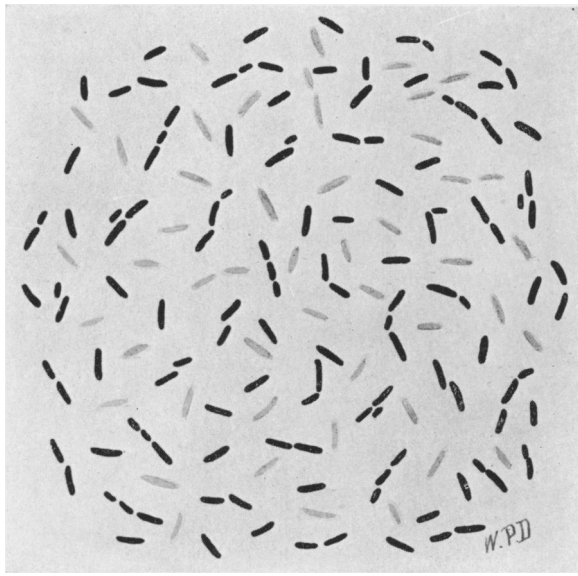

FIG. 20

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

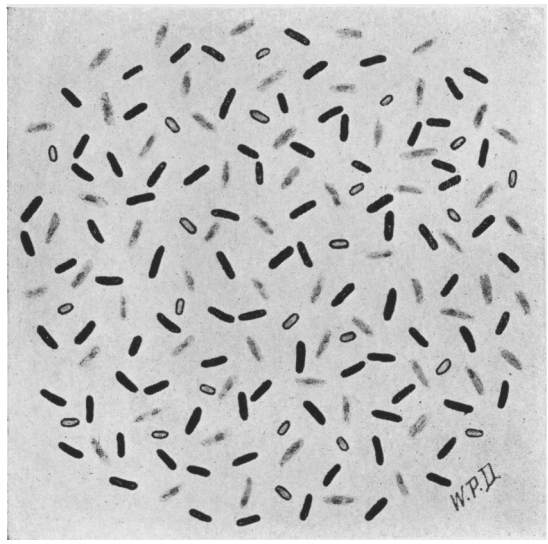

FIG. 21

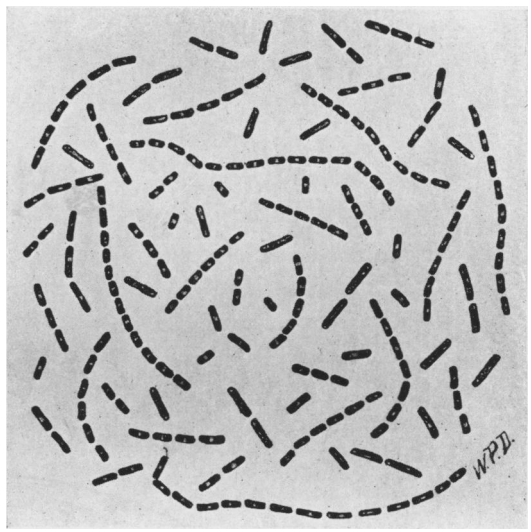

FIG. 22

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

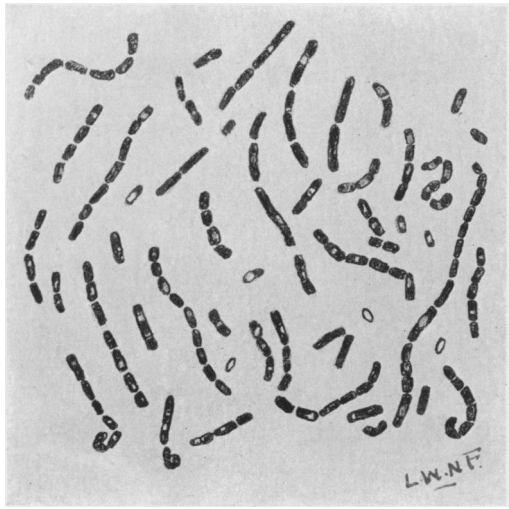

FIG. 23

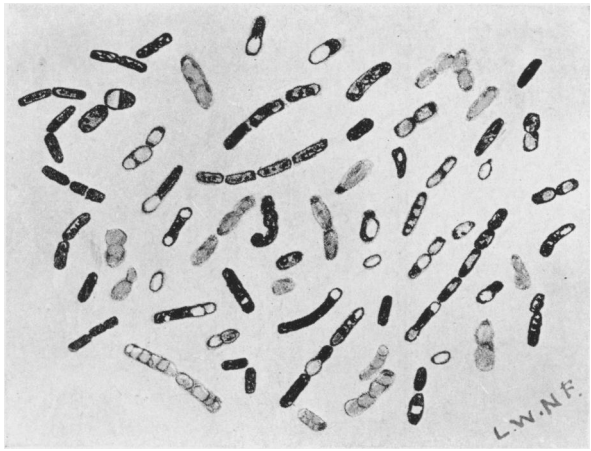

FIG. 24

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

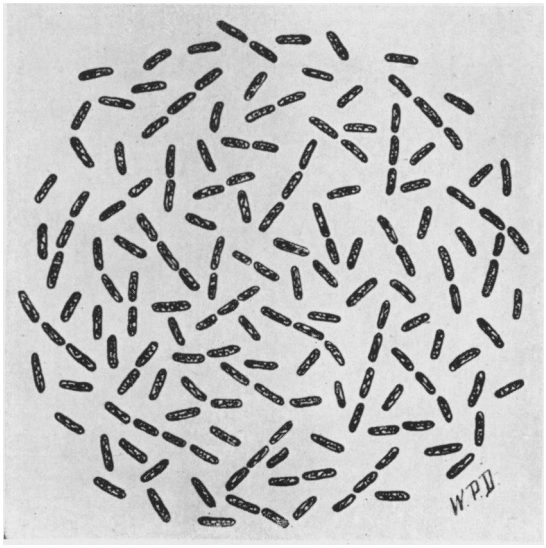

FIG. 25

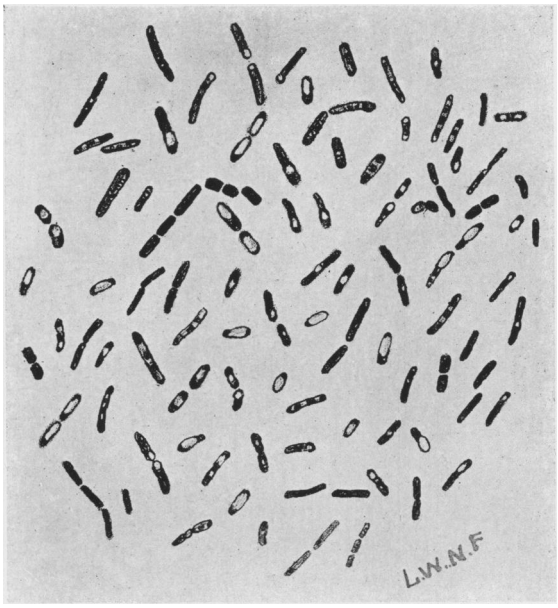

FIG. 26

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

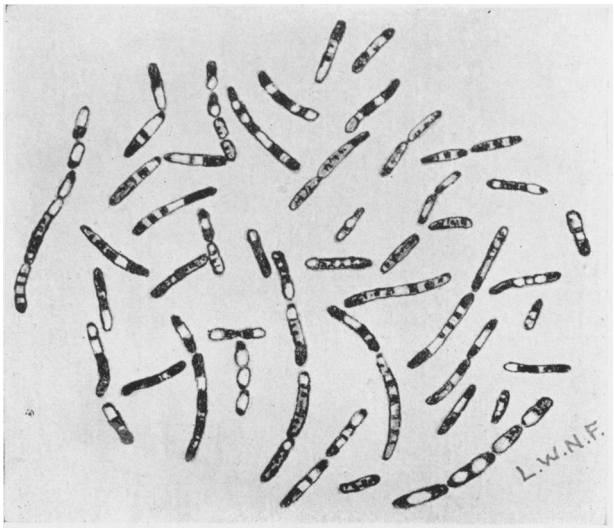

FIG. 27

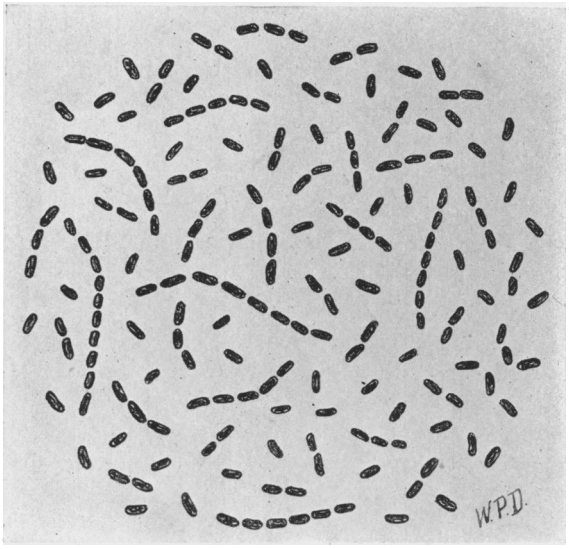

FIG. 28

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

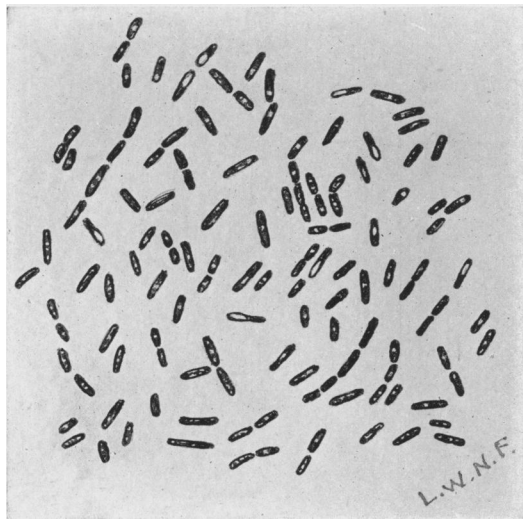

FIG. 29

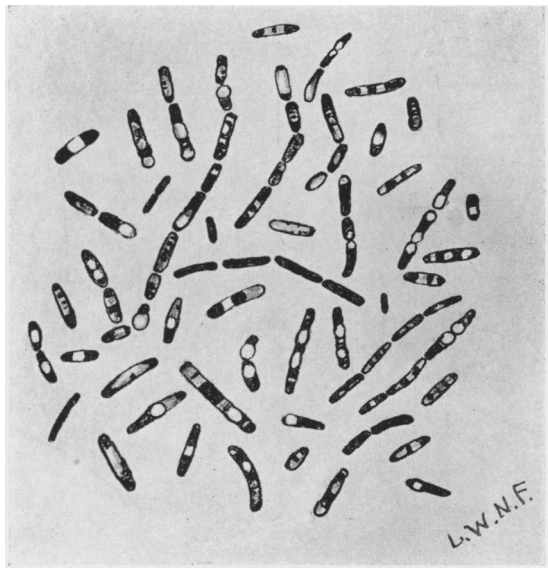

FIG. 30

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

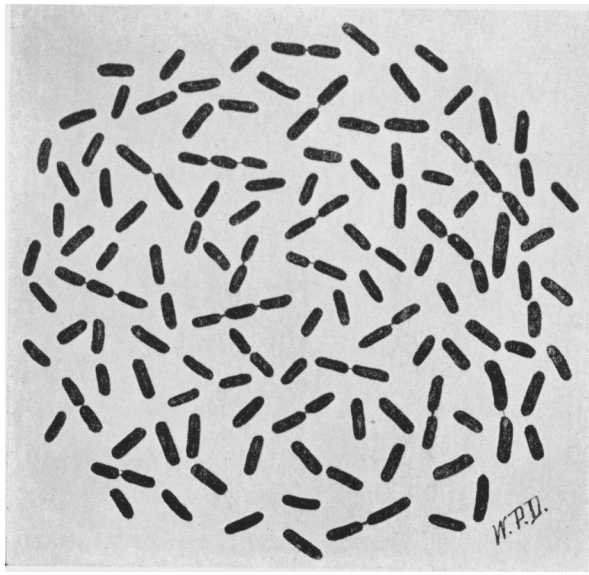

FIG. 31

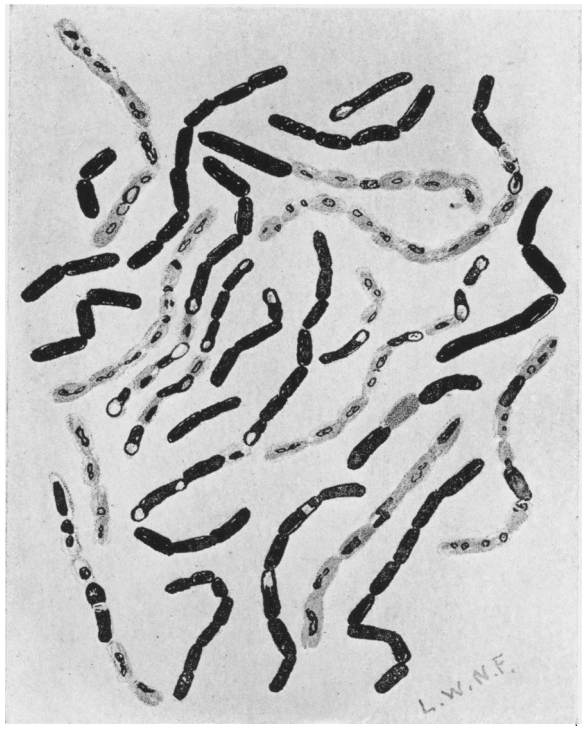

FIG. 32

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

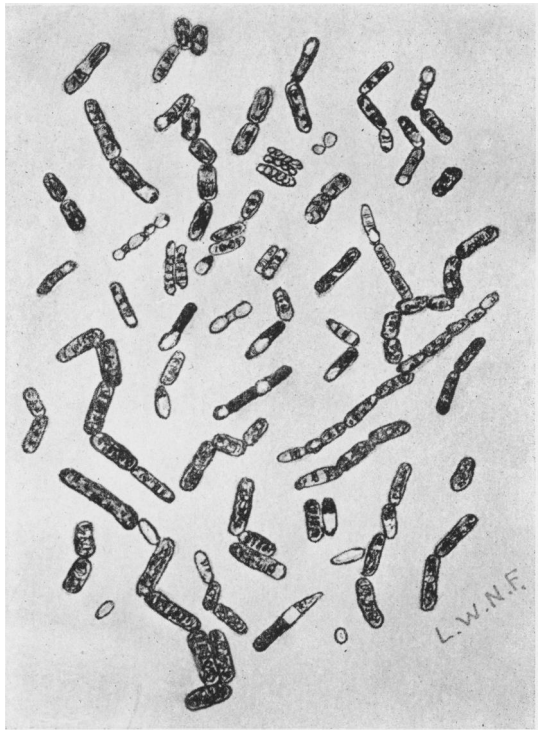

FIG. 33

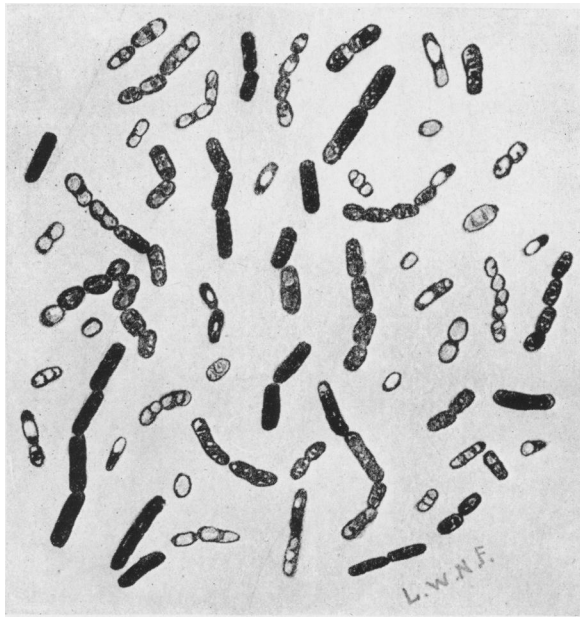

FIG. 34

Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

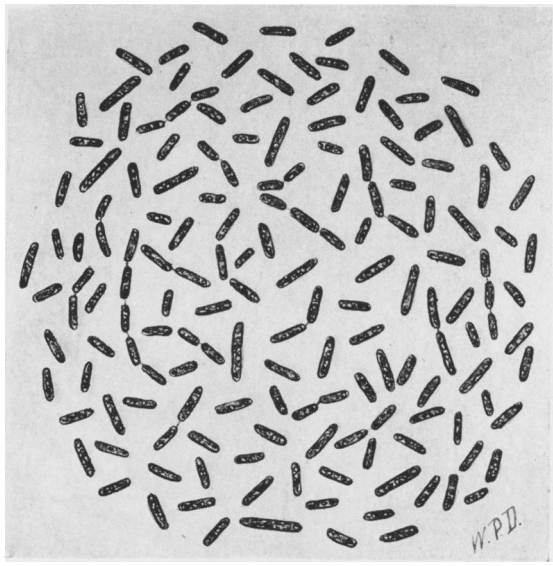

FIG. 35

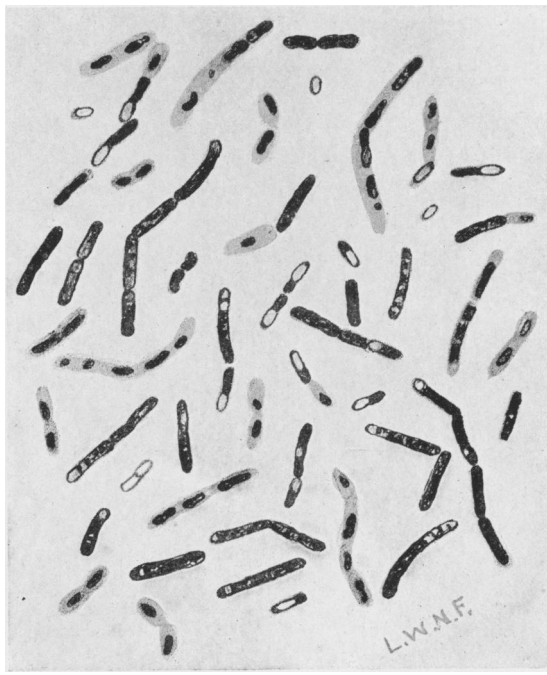

FIG. 36

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria

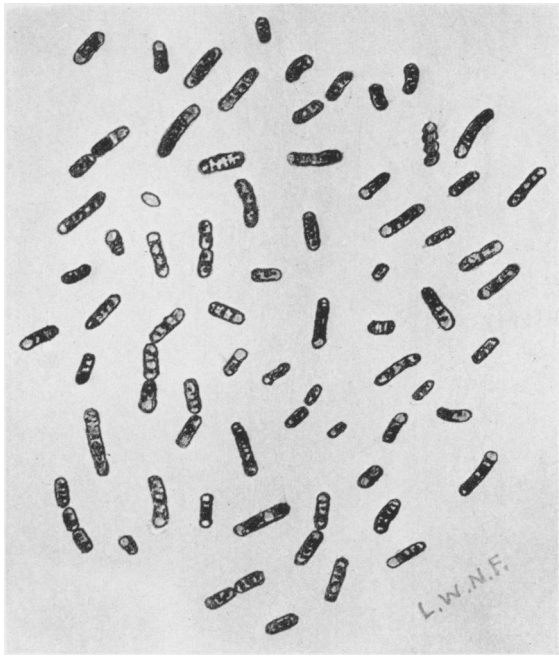

FIG. 37

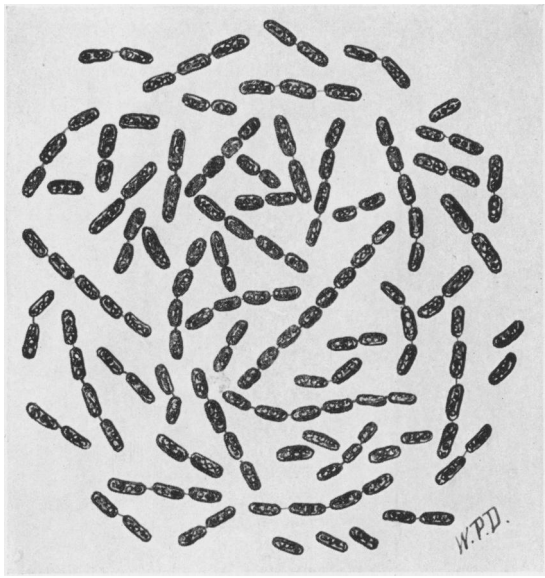

FIG. 38

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

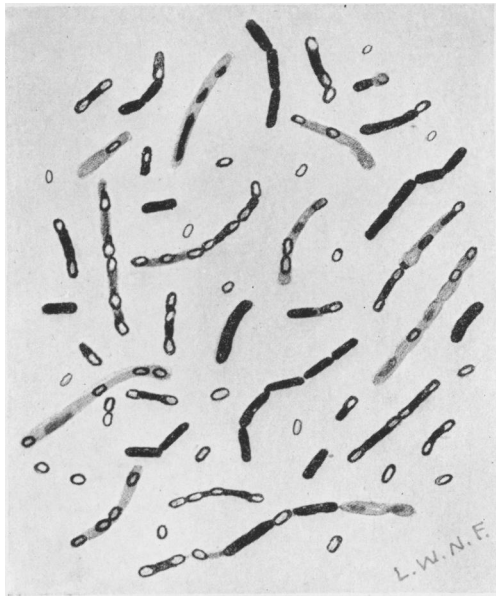

FIG. 39

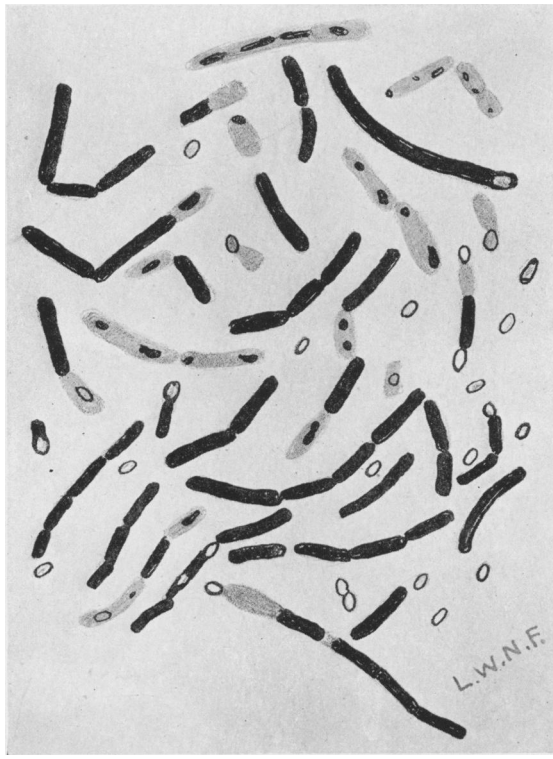

FIG. 40

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

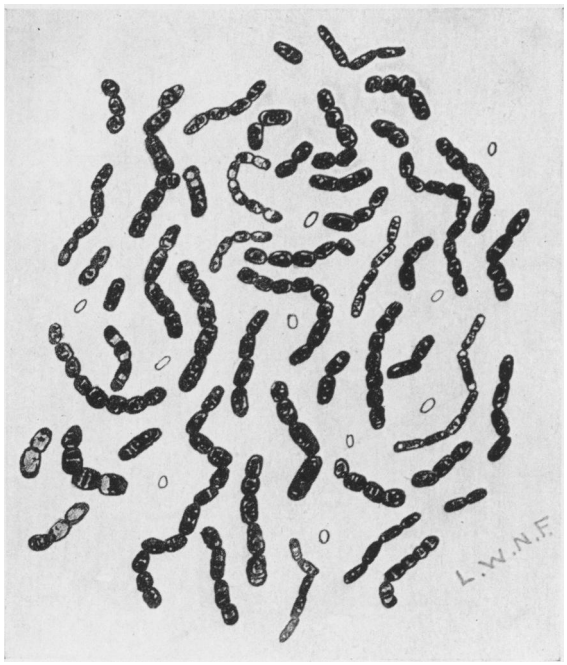

FIG. 41

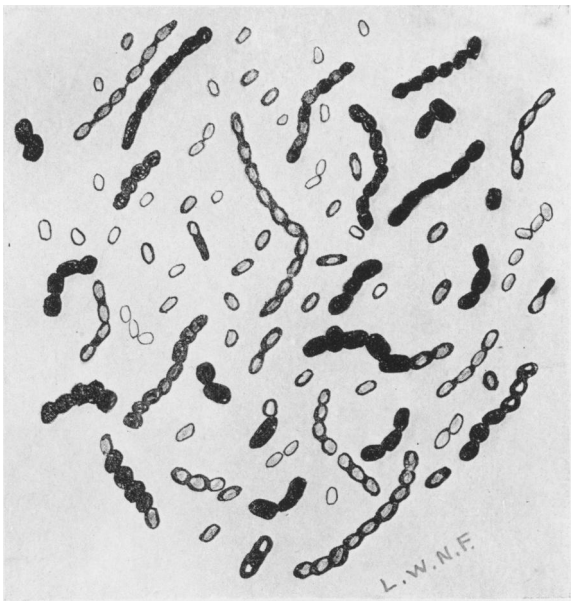

FIG. 42

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

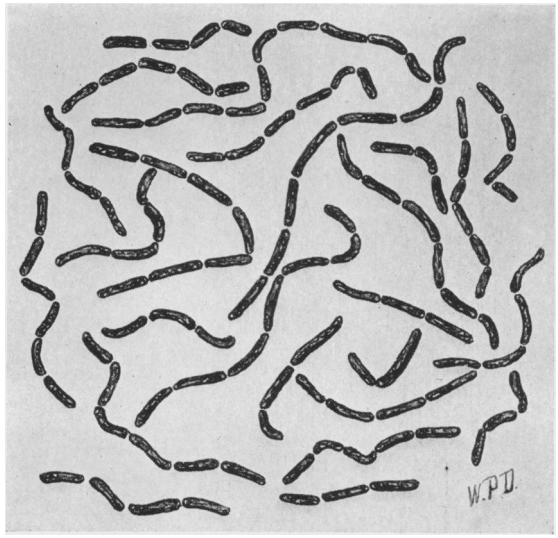

FIG. 43

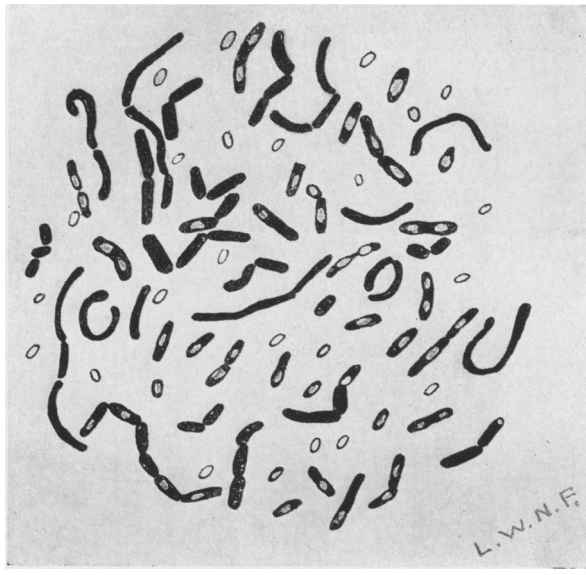

FIG. 44

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

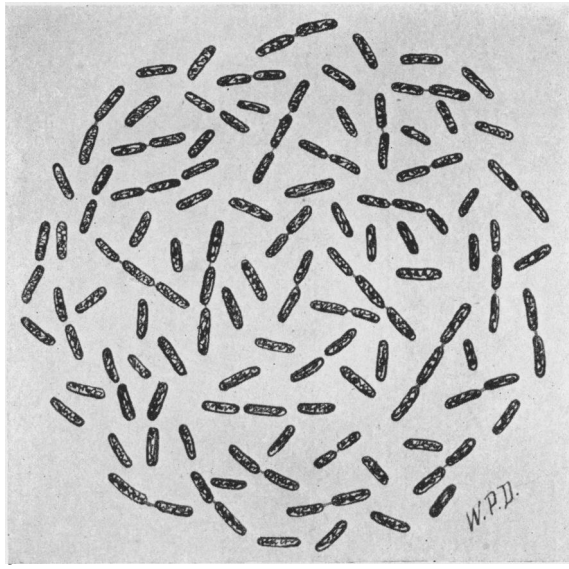

FIG. 45

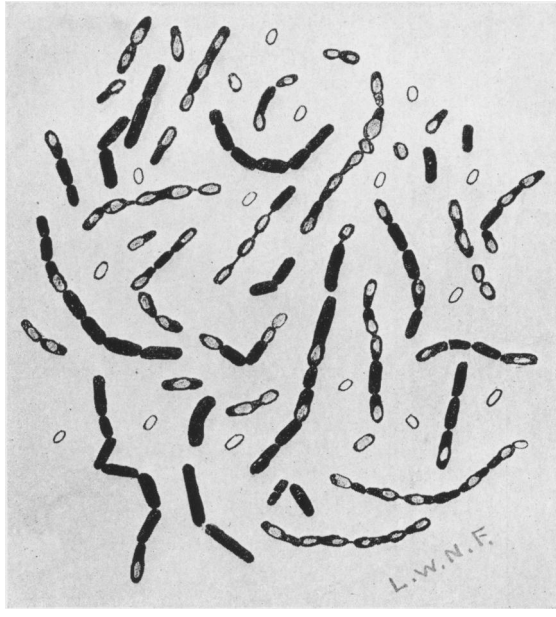

FIG. 46

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

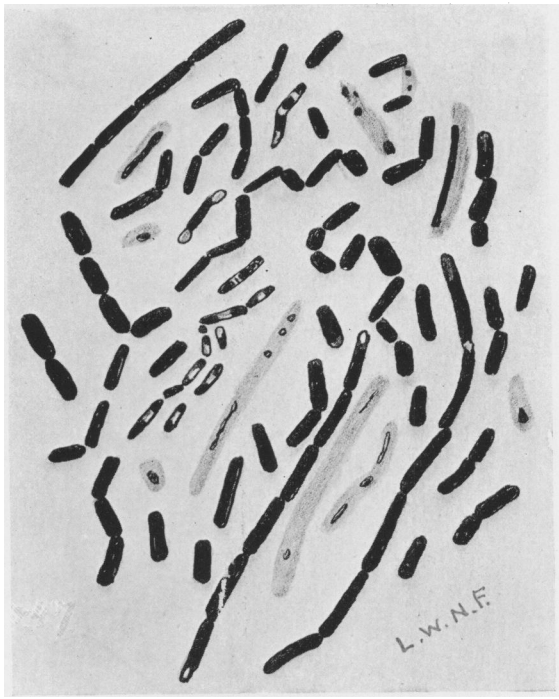

FIG. 47

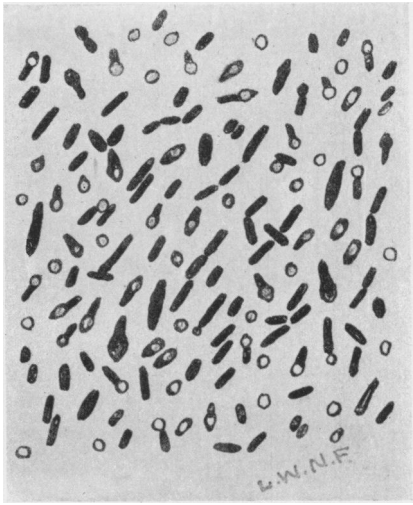

FIG. 48

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

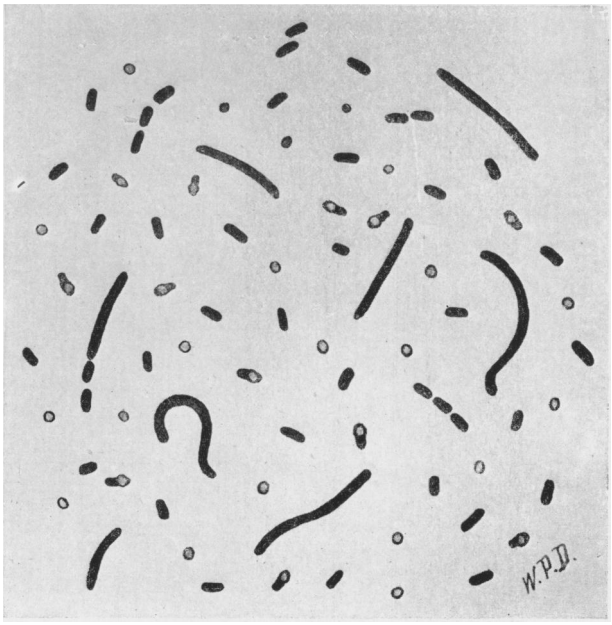

FIG. 49

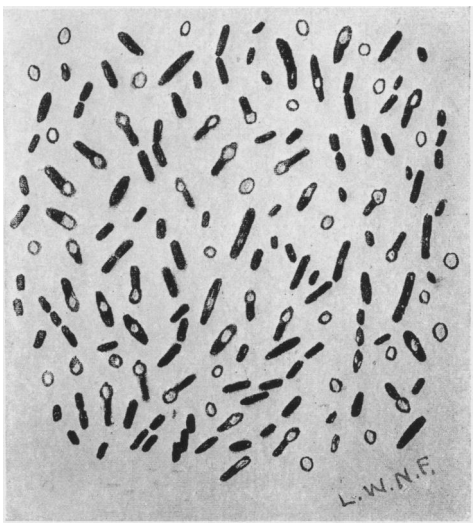

FIG. 50

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)

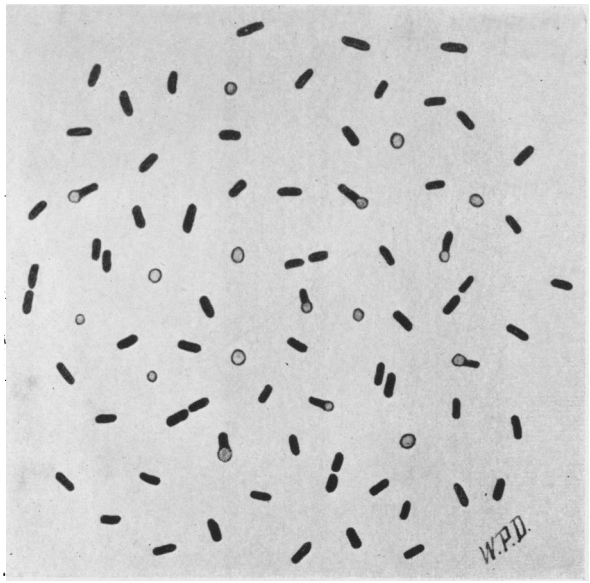

FIG. 51

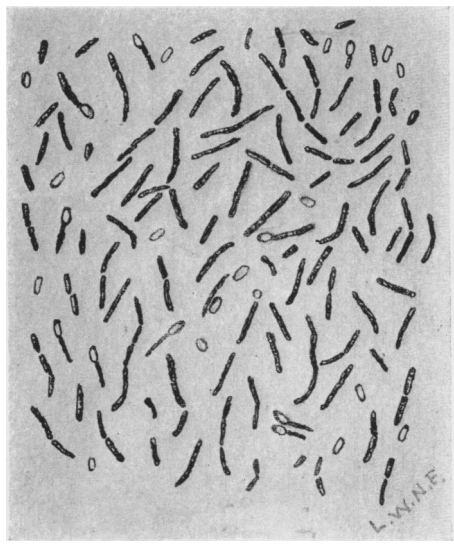

FIG. 52

(Lawrence and Ford: Aerobic Spore-bearing Non-pathogenic Bacteria)