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PLEOMORPHISM AND PLEOBIOSIS OF BACILLUS BIFIDUS COMMUNIS.*

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PLATES X–XIII.

Notwithstanding the extensive studies of Tissier,[1] Moro,[2] Rodella,[3] Cahn,[4] Passini,[5] Jacobson,[6] Herter and Kendall,[7] and a few others,[8] the relation between *B. bifidus communis* (Tissier, 1900) and certain other Gram-positive organisms of the feces, such as *B. acidophilus* (Moro, 1900), Kopfchenbacillus, *B. tuberculiformis intestinalis* (Jacobson, 1908) and *B. infantilis* (Kendall, 1909) remains still unsolved. At present it is difficult to say whether they are entirely different organisms, or whether they are different forms of one and the same organism. It is a recognized fact that the purity of a culture of *B. bifidus* cannot be guaranteed absolutely, as it often produces forms which differ in many respects from the original strain and which resemble, not infrequently, *B. acidophilus, B. tuberculiformis intestinalis,* or the Kopfchenbacillus. Moro abandoned his effort to identify the Kopfchenbacillus with *B. bifidus*

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[1] Tissier, *Compt. rend. Soc. de biol.,* 1899, li, 943; Recherches sur la flore intestinale des nourrissons, Paris, 1900; *Ann. de l'Inst. Pasteur,* 1905, xix, 109.

[2] Moro, *Jahrb. f. Kinderh.,* 1900, lii, 38; *Wiener klin. Woch.,* 1900, xiii, 114; *Jahrb. f. Kinderh.,* 1905, lxi, 687.

[3] Rodella, *Cent. f. Bakt, I Abt., Orig.,* 1901, xxix, 717; *Zeit. f. Hyg.,* 1902, xxxix, 201; *Cent. f. Bakt, I Abt., Orig.,* 1903, xxxiv, 14.

[4] Cahn, *Cent. f. Bakt, I Abt., Orig.,* 1901, xxx, 721.

[5] Passini, *Jahrb. f. Kinderh.,* 1903, lvii, 87.

[6] Jacobson, *Ann. de l'Inst. Pasteur,* 1908, xxi, 303.

[7] Herter and Kendall, *Jour. of Biol. Chem.,* 1908, v, 289; Kendall, *Jour. of Biol. Chem.,* 1909, v, 419.

[8] Finkelstein, *Deutsche med. Woch.,* 1900, xxvi, 263; Cipollina, *Cent. f. Bakt, I Abt., Orig.,* 1902, xxxii, 576; Weiss, *Cent. f. Bakt, I Abt., Orig.,* 1904, xxxvi, 13.

because of his inability to produce a bifurcating strain from the former, while Jacobson met a similar failure in his attempt to reverse *B. tuberculiformis intestinalis* into *B. bifidus.* In spite of numerous attempts, as yet no one has succeeded in tracing the source of *B. bifidus* outside of the intestinal tract.

In the course of my study of anaerobic bacterial flora of the intestinal tract of healthy and diseased children, my attention was directed to *B. bifidus.* The results I obtained from my present study on *B. bifidus* convinced me that *B. bifidus communis* of Tissier is an anaerobic phase of life of an aerobic sporogenous organism belonging to the subtiloid group and closely resembling, especially morphologically and biologically, *B mesentericus fuscus.* By certain cultural methods, I was able to induce sporulation and adaptation of the aerobic life of *B. bifidus* and then lead the aerobized *B. bifidus* back to the anaerobic bifurcating phase. In the aerobic phase of *B. bifidus,* no bifiurcation has been observed, and it seems almost incredible that it should be related to anaerobic *B. bifidus* at all. With adaptation of aerobiosis and anaerobiosis the entire sets of morphological and biological characteristics undergo profound alterations. There are intermediate phases between these two extremes, which give the bacillus characteristics of semi-anaerobiosis and extreme morphological variabilities.

The following experiments were made. Eight pure colonies of *B. bifidus,* two of which consisted of a somewhat more delicate type of bacillus, and one pure colony of a bacillus resembling a type of *B. acidophilus* were isolated from the fresh stool of a healthy, breast-fed girl, aged two months, by means of glucose-agar plating in an anaerobic apparatus constructed on the same principle as that of Schattenfroh and Grassberger. The purity of these colonies was ascertained first by microscopical examination and then by culture. For the latter purpose, I found it advisable to use high layer agar containing 1.5 per cent. lactose or glucose, because with this bifurcation was more general and uniform than with any other liquid media for which an anaerobic apparatus is required. Even with the use of fresh tissue in bouillon or glucose bouillon, *B. bifidus* was seen to bifurcate less constantly than in high layer sugar agar,

and to produce a pleomorphic condition[9] which makes it difficult to determine the purity of an organism. The bacillus which appeared like *B. acidophilus* in colony turned out in high layer glucose agar to be a regular bifurcating strain. On the other hand, a typical bifidus strain often grew out of colonies with some bifurcating and non-branching acidophilus types, giving the appearance of mixed colonies.

SPORULATION OF B. BIFIDUS COMMUNIS.

Inoculations with these nine strains were made into solid and liquid media. The organisms were inoculated in the melted state, or by stab, into the high layer agar containing lactose or glucose (1.5 per cent.) and were cultivated at 37° C.; the inoculations into liquid media (litmus milk, beer wort bouillon, glucose bouillon, and especially Hiss's serum water, containing different sugars) were cultivated usually for seven days before examination in an anaerobic apparatus at 37° C.

The agar cultures were examined from time to time and in most cases I found bifurcation within twenty-four hours. As the bacilli grew older, the number of bifurcations and the length of the branches increased, and at the same time lost their ability to stain with Gram's method (Figs. 1, 2, 3, 4, 5).

The lactose- and glucose-serum-water were suitable media for *B. bifidus* and the sugars were fermented, without gas production, up to coagulation of the serum (acid production). Microscopical examination showed the presence of pleomorphic, non-branching forms (diplobacilli with tapering ends, spindles, short rods, beaded forms, coccobacilli, and a few bifuracted specimens). Vesicular forms were also seen occasionally. Besides these, I observed occasional free or attached oval spores (Figs. 6, 7, 8, 9).

By cultivating in succession pure strains of *B. bifidus* in sugar containing agar by my modification of the Marino plate,[10] I was

[9] There are in such cultures usually non-branching forms, including delicate bacilli with tapering ends, club-shaped, beaded, or tad-pole forms. Bifurcation is rare. In beer wort bouillon, delicate bifurcation may sometimes be produced.

[10] Marino, *Ann. de l'Inst. Pasteur*, 1908, xxi, 1005. I made two narrow breaks on opposite sides of a small Petri dish. I then placed it, with the opening downwards, in a larger Petri dish so that the liquid agar could flow from the large dish into the smaller one.

able to obtain, at intervals of two or three weeks, colonies in which a few straight Gram-positive bacilli were seen side by side with Gram-negative, bifurcated forms apparently of a degenerating stage. A few spores were often seen in such cultures (Figs. 10, 11, 12, 13).

The spore-forming organisms of these old cultures of *B. bifidus* were isolated by the usual methods. The cultures were heated to 100° C. for five minutes and plated out with modified Marino plates or diluted in high layer agar containing lactose and glucose.

From each strain I obtained two distinct strains of bacilli, which differed in their sensitiveness to oxygen, and also slightly in their morphological and cultural characteristics. One of the two was strictly aerobic, while the other was a facultative anaerobe. The first produced felted, dry colonies and contained a spore-forming, Gram-positive, motile bacillus, while the second formed rather moist, greyish-white colonies and the bacilli were somewhat more slender than those of the first. I found, however, that the second variety becomes gradually strictly aerobic and like the first variety after a few successive aerobic cultivations (Figs. 14, 15, 16, 17).

In the following protocols, I have given detailed descriptions of these two varieties of bacilli which have sprung from apparently pure cultures of *B. bifidus* under certain circumstances. The morphology of these two strains will be given in Table I which follows the experiments on reversion.

CULTURAL CHARACTERISTICS.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
Agar plate plain agar.	24 hours at 37° C.—The colonies are very small, irregularly round or oval, and rather opaque. Diameter less than 1 mm. Under low power magnification: edge fairly well defined with curled filaments projecting from the entire circumference, some as long as the diameter of the colony. These curled filaments are irregularly interwoven, but sparse. Struc-	24 hours at 37° C.—The colonies are round or oval, irregularly contoured, elevated, finely granular, faintly grayish-brown, dimly edged, opaque, some containing thick centers. Deep colonies roundish or lenticular. By reflected light, grayish-white and shiny. Diameter of single colonies about 1 mm. 4 days at 37° C.—*A felted edge* is formed around the colonies

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	ture: yellowish-brown, granular appearance due to the interwoven curved filaments without distinctive nucleus. By reflected light the colony looks grayish-white, dry, raised, and of irregular surface. 72 hours at 37° C.—The colonies are about 6 mm. in diameter, and show typical felted appearance.	without changing smoothness of the latter.
Glucose agar.	24 hours at 37° C.—The size of colonies is somewhat larger than that of plain agar colonies. Irregularly round or oval. Opaque, yellowish-brown and darker than in plain. Edge irregularly serrated, fairly well defined, and projecting from it many uneven, almost transparent, finely granular, wart-like outgrowths. Outer contour is surrounded by a lamellated marginal zone. This peripheral zone is caused by mucilaginous secretion of the colonies. By reflected light the colonies look grayish, pulvinated, with dried edge and moist center, which give a pearl-like appearance. Corrugated structure. 72 hours at 37° C.—Many colonies sent out typical felted filaments.	24 hours at 37° C.—Much the same as colonies on plain agar, but there is mucin production and often the colonies have a felted edge of long filaments comparable with the aerobic phase colonies. 4 days at 37° C.—Growth more vigorous but no other essential change.
Agar stab plain agar.	24 hours at 37° C.—Filamentous growth which is better nearer the surface. 48 hours.—Slight outgrowth along the stab near the surface. 7 days.—Growth covered the surface with thick membrane. 30 days.—Browned the media.	24 hours at 37° C.—Filiform growth along entire stab. There is a slight surface growth out of the puncture. 48 hours.—Slight surface growth. 7 days.—No further change. 30 days.—Browned the media slightly.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain)
Glucose agar.	24 hours at 37° C.—Thin, uniform, smooth, filiform growth. Surface outgrowth is abundant. 48 hours.—Little outgrowth near the surface. Thick, mucoid, grayish membrane covered the surface. 30 days.—Browned the media.	24 hours at 37° C.—Similar to the above. 30 days.—Slightly browned the media.
Melted agar—inoculation.	24 hours at 37° C.—Growth obtained only on the surface, none in the deeper layer.	24 hours at 37° C.—Small punctiform colonies throughout the entire agar column. No gas formation. Surface is covered with thin layer of colony.
Plain agar.	After a few days the colony became thick, grayish, wrinkled, tough. 30 days.—Some root-like colonies grown into the upper layer of the agar. Browned the agar.	30 days.—Apparently no change, except browning the media.
Glucose agar.	Similar to the above, but more mucoid in character. 30 days.—Browned the agar.	24 hours at 37° C.—Somewhat better growth in the layer nearer the air. 30 days.—Apparently no change, except browning the media.
Agar stroke plain agar.	24 hours at 37° C.—Abundant, broad, spreading, rather flat, slightly more elevated in the middle, and firmly adherent, rugose, opaque, felt-edged colony. Grayish-white, in general dry, but beset with minute glistening droplets. Not larger than 1 mm. By transmitted light, the margin and center distinctly defined, the margin being dense and opaque—about 4 mm.; the center is less opaque and is finely wrinkled. Condensed water is slightly turbid and has thin, imperfect, grayish-white film. Medium unchanged.	24 hours at 37° C.—Moist, grayish-white, smooth-edged, but quite zigzag near the condensed water, where it spreads. Condensed water is turbid, *without* a film. Medium unchanged. 4 days at 37° C.—The edge of the colony assumed a zigzag appearance.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	72 hours at 37° C.—Growth advanced.	
Glucose agar.	24 hours at 37° C.—Spread over all surface. Raised by the accumulation of mucoid droplets which are almost transparent. Entire surface is covered with mucus drops of varying sizes. Medium unchanged. Colonies difficult to scrape from agar. 72 hours at 37° C.—Began to present more felted appearance and to show a tendency to get dry.	24 hours at 37° C.—Spreading, grayish white, undefined edge with numerous outgrowths projected from it. There is tendency to confluence. The colony is rather flat and very slightly elevated. The condensed water is cloudy. Medium somewhat turbid. 4 days at 37° C.—Slowly growing, but no felted appearance developed. The colonies do not adhere to the agar firmly, but can easily be removed with loop. The consistence is sticky.
Litmus milk.	24 hours at 37° C.—Color turned red, and a heavy yellowish-white deposit is formed on the bottom. 48 hours.—More yellowish. 72 hours.—Coagulated. 30 days.—Much of the coagulated casein dissolved. Reaction is slightly acid.	24 hours at 37° C.—Reddened on the top, and deeper layer decolorized. 7 days.—Coagulated. 30 days.—Coagulation seems to have dissolved somewhat. The reaction remains acid.
Loeffler's serum.	24 hours at 37° C.—Grayish-white, rugose, spreading colony. The substance beneath the colony appears to be depressed. There are mucoid droplets on the colony. 48 hours.—Liquefaction already set in and advancing. 4 days.—Not completely liquefied. 30 days.—Lower portion of the medium almost entirely dissolved. Brownish.	24 hours at 37° C.—Whitish-gray, shiny, elevated, wavy edged, no depression of the medium. 48 hours.—No definite liquefaction. 4 days.—No further change. 30 days.—Somewhat sunken along the colony, indicating a feeble power of proteolysis.
Gelatin.	24 hours at 37° C.—Minute flocculi in clear medium. On the surface are floating grayish-	Much the same as the aerobic phase, but the liquefaction is decidedly slower.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	white, dull, irregularly contoured pieces of colonies. 72 hours at 37° C.—Scum is formed. 7 days.—Liquefaction apparent. At 20° C.—Very limited amount of liquefaction of gelatin along the stab puncture in 2 weeks. 30 days.—Moderate liquefaction. Slight surface growth. In the stab there are many lateral projections of fine growth near the surface. Liquefaction is about 1 mm. deep and 6 mm. in diameter.	At 20° C.—Liquefaction is slight after 30 days. Growth is also poorer.
Plain bouillon.	24 hours at 37° C.—Slight turbidity with strong surface membrane, which sinks when torn, with the exception of narrow annulæ around the surface on the tube-wall. On vigorous shaking it breaks up into a fine flocculence. 48 hours.—No new scum. 72 hours.—A new, firm, wrinkled, whitish-gray membrane over the surface.	24 hours at 37° C.—Slightly turbid, with slight whitish sediment. No surface membrane. 72 hours.—No further change, except more turbidity and deposit.
Glucose bouillon.	24 hours at 37° C.—More turbid and finely granular sediment than in plain bouillon. On shaking, the sediment diffuses. No surface membrane except the annula. 48 hours.—Distinctly acid. 72 hours.—Firm surface membrane formed.	Similar to the above. Some acid production. No scum.
Potato.	24 hours at 37° C.—Grayish-white (faintly brownish at the margin of the colony), wrinkled, thick, scaly, dull, spreading. The colony is adherent to potato, but is quite	24 hours at 37° C.—Growth on potato *invisible,* with the fluid very turbid. 4 days.—No visible surface growth. 30 days.—No further change.

	Aerobic Phase. (1st Strain.)	Semi-Aerobic Phase. (2d Strain.)
	moist and brittle (suggestive of colonies *B. tuberculosis* on veal bouillon-glycerin agar slant). 48 hours.—Color intensified and colony thicker and spreading. 72 hours.—Distinctly brownish-gray, somewhat reddish in places. Thickened and heavily wrinkled. Scum on the fluid. 4 days.—Brownish and dirty hue. 30 days.—Dark, brownish, gray hue.	

The descriptions given of the aerobic strain of the two organisms agree closely with those of *B. mesentericus fuscus.*

REVERSION OF AEROBIC PHASE OF B. BIFIDUS COMMUNIS INTO ANAEROBIC PHASE.

In the foregoing pages I described "the springing out" of two varieties of spore-forming, non-branching aerobes from certain cultures of *B. bifidus.* This finding might, of course, be considered as a gross contamination with the brown potato bacillus, had I not been able to produce all typical vegetative forms characteristic in every detail of *B. bifidus communis* from the spore material heated to 100° C. for five minutes of these two strains. However, by gradual training of the organisms to anaerobic life, I was able to accomplish complete reversion of the aerobic phase of this bacillus into the anaerobic. I cultivated the bacilli first semi-aerobically, abruptly diminishing the quantity of oxygen. After three or four successive cultivations, I obtained anaerobiosis in which condition the organism had grown well. Glucose bouillon and Hiss's serum water containing different carbohydrates, especially lactose, dextrin, inulin, saccharose and amygdalin, were found to be very suitable for reversing the biological phase. Anaerobic condition was produced usually by hydrogen gas, although nitrogen, carbon dioxide and methan were equally suitable for the production of typical bifurcating forms. The degree of reversibility of the strictly

aerobic variety is found to be inferior to that of the semi-aerobic strain, and more generations are required before complete reversion is attained.

During the reversing processes, I observed that the first step of reversion of the aerobic spore-material towards anaerobiosis is characterized by the appearance of streptococcal or young staphylococcal forms with, occasionally, *minute* forms of bifurcating types. In the next stage the numbers of coccobacillary forms gradually diminish and more typical bifurcating bacilli of *regular* size and shape, with many short primitive types of bifidus, appear. In this stage, the developmental steps from coccal forms to regular bifidus are clearly seen (Figs. 18, 19). Stellated arrangements of young bifurcating rods are often seen.

A further step towards anaerobization shows the tendency to form more bifurcated bacilli of regular size, and in the stage following this phase, we obtained *B. bifidus communis* in the sense used by Tissier (Figs. 20, 21, 22).

The fermentative faculty of *B. bifidus* and its aerobic varieties were tested upon different kinds of sugars and glucosides, but the results were extremely inconstant. In one series of experiments they split almost every sugar employed with acid production and, on other occasions, they did not attack any of them. I found, however, that in the strictly aerobic phase of the bacillus it is rare to get coagulation of serum or milk, while in semi-aerobic and strictly anaerobic phases, acid-production seems to cause coagulation of these proteids in several days. This difference may be due to simultaneous proteolysis in the aerobic phase. Table I describes summarily the morphology of the four different phases of *B. bifidus.*

CONCLUSIONS.

From the foregoing experiments, the conclusion may be drawn that *B. bifidus communis* of Tissier has an aerobic phase, in which it resembles *B. mesentericus fuscus.* Numerous intermediate phases can occur between these two extremes; and their morphological and biological variabilities demand the utmost attention in order to interpret more intelligently the various phases of a given organism, constantly found in the stools of sucklings, and to avoid the artificial creation of two or more organisms from a single microbic type.

TABLE I.

MORPHOLOGY.

	Size.	Chains.	Threads.	Pairs.	Motility.	Sporulation.	Gram's Stain.
Aerobic phase (obligatory). The 1st strain belongs to this phase.	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ.	More frequent in bouillon, some covering entire field. In old cultures more than in the new.	Rare, but may be seen in old liquid culture, often quite long, equalling half the field. 20 to 50 bacilli may be seen. Almost none in young agar culture.	2, seldom 3 or 4, parallel, occur quite frequently.	Very active serpentine, rotating, waltzing. Flagella at both ends.	Occurs within 24 hours in almost every case. Polar or middle spores, oval, spore membrane noticed, especially at the antipole. Easy detachment from the bacilli. Length, 0.8μ to 1.5 μ. Width, 0.5 μ to 0.8 μ.	Young cultures usually uniformly positive. After 2 to 4 days the staining becomes less intense and decolorized more easily by alcoholic treatment.
Facultative anaerobic phase (tendency toward aerobic). The 2d strain belongs to this phase.	Length, 2 μ to 5 μ. Width, 0.3 μ to 0.6 μ.	There are very few branching forms. There are forms of straight, round ended, or somewhat curved diphtheria bacillus-like forms Some give the appearance of tubercle bacilli.		Pairs are often present.	Some are quite actively and many are slowly motile. Many dancing, waggling and rotating. Flagella at both ends.	Early sporulation occurs in the straight forms, but only in very old cultures does it occur in other types. Almost always polar. Shape and size same as above. (Fig. 16.)	There are various specimens with different degrees of staining by Gram's method. The straight forms stain more intensely.
Facultative anaerobic phase (tendency toward anaerobic).	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ. In reversing experiments from the spores coccobacillary forms or punctiforms often appear. They are much smaller in size and more pleomorphic than the phase arising from the anaerobic bifidus. (Figs. 17 and 18.)	Geniculated non-branching forms predominate. The ends may be pointed or thickened to suggest the bifurcation. There may be a few branching forms resembling tubercle bacilli or dotted bacilli like diphtheria bacilli.		Pairs of non-branching forms are frequent.	Some slowly motile. Others immobile.	Fewer or no spores are produced by the young cultures, but almost always some are formed in the old semi-aerobic cultures. Always polar. Shape and size same as above.	Young cultures quite uniform; sometimes complete decolorization in a short time.

	Size.	Chains.	Threads.	Pairs.	Motility.	Sporulation.	Gram's Stain.
Anaerobic phase (obligatory) or *B. bifidus communis* of Tissier.	Length, 1.5 μ to 5 μ. Width, 0.3 μ to 0.6 μ. From a continuous bunch of bifurcated bacilli, in old lactose agar, one projection measured about 10 μ.	Geniculation of branching forms twice or many times is common in old solid cultures. There are examples of sternata arrangement of bifurcating forms. This is more frequent in the reversing of spores into anaerobic phase. Straight forms may appear in old sugar agar cultures.		In young, non-branching cultures, 2 to 4 or even more, parallel to each other, occur. Bifurcating forms do not pair so often.	Motility doubtful, perhaps absent.	While young, there is no sporulation, but spores appear in straight bacilli in old cultures under semi-anaerobic preservation. Always polar, often free. Shape and size same as above.	Young cultures quite uniform; the bifurcating portion of branches decolorize much more easily than the other parts. Gram's stain becomes irregular when cultures get old, and may decolorize completely.

The frequent occurrence of *B. mesentericus* in the stools of sucklings has been described by Tissier, Moro, and many others. That *B. mesentericus* is one of the most wide spread saprophytes and is constantly found on the surface of our skin, is a well established fact. Scheurlen[11] was once led to consider this bacillus as the cause of carcinoma, because he isolated it from the cancers of mamma (interior of the tumors). But Rosenthal[12] found the same organism in the breasts of healthy persons.

I, therefore, consider that the one source of *B. bifidus communis* in the stools of breast-fed infants is the breast of the lactating mother.

EXPLANATION OF PLATES.

PLATE X.

FIG. 1. Non-branching forms of *B. bifidus communis.* From a young culture in high layer glucose agar.

FIG. 2. The beginning of bifurcation of *B. bifidus.* (18-hour-old glucose agar stab culture.)

FIG. 3. Pleomorphic state of young *B. bifidus* culture, showing the tridents, *Y*-forms, wedges, geniculations, non-branching individuals, etc. From a young, lactose agar high layer culture (48 hours old).

FIG. 4. Multiple bifurcations of *B. bifidus.* From an old, high lactose agar culture.

FIG. 5. Multiple bifurcations of *B. bifidus* showing abnormally large individual with enormously long projections. From an old, high glucose agar culture.

FIG. 6. Sporulation of *B. bifidus.* A spore-bearing short bacillus with two individuals is seen on the left. Straight, curved, or geniculated forms are also seen, but very few bifurcated forms. This shows the stage of springing out of aerobic individuals from an old culture of *B. bifidus.* From an old inulin-serum water culture.

PLATE XI.

FIG. 7. Non-branching phase of *B. bifidus.* Diplobacilli forms, candle-flame forms, banded and striated forms and many other forms are seen. From 7-day old lactose serum water culture.

FIG. 8. Pleomorphic feature of *B. bifidus.* From a young saccharose serum water culture.

FIG. 9. Sporulation of *B. bifidus.* Besides Gram-negative bifurcated forms, there are several Gram-positive straight bacilli which represent the semi-aerobic phase of *B. bifidus.* From an old culture in modified Marino plate.

FIGS. 10 and 11. Sporulation of *B. bifidus.* From a 14-day-old lactose agar plate after modified Marino method.

[11] Scheurlen, *Berliner klin. Woch.,* 1887, xxiv, 935.

[12] Rosenthal, *Zeit. f. Hyg.,* 1889, v, 161.

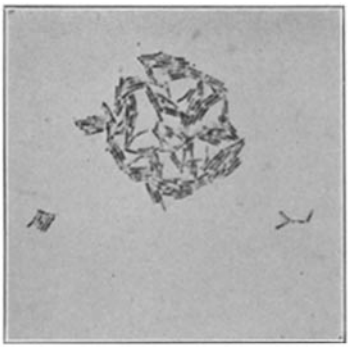


FIG. 1.

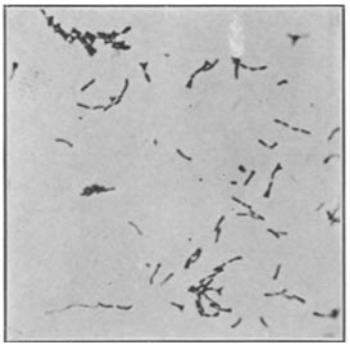


FIG. 2.

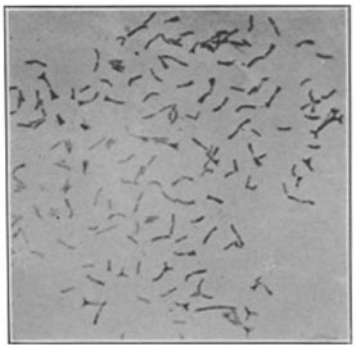


FIG. 3.

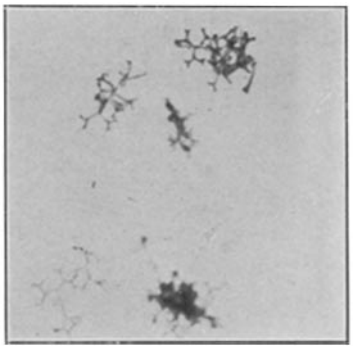
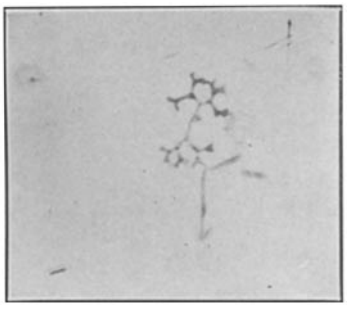


FIG. 4.



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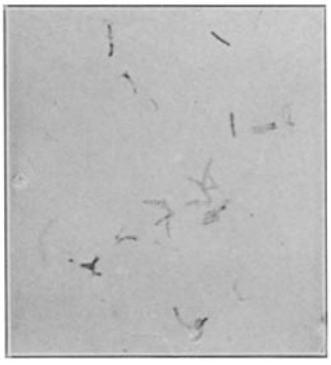


FIG. 6.

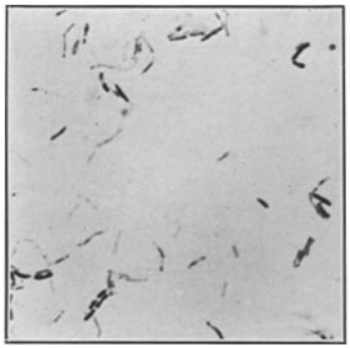


FIG. 7.

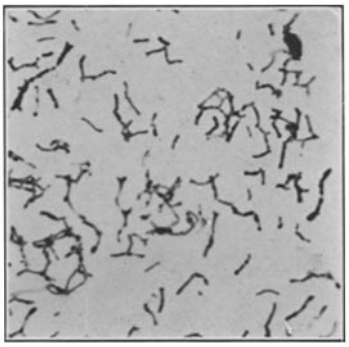


FIG. 8.

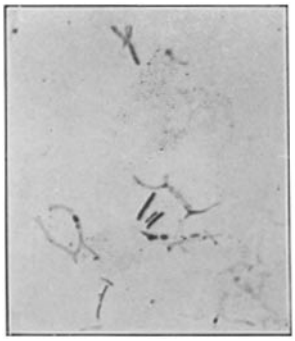


FIG. 9.

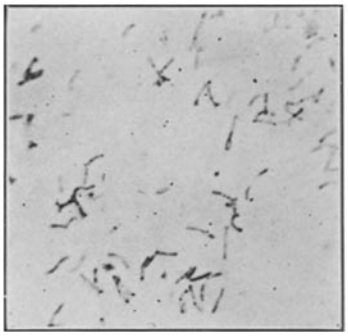
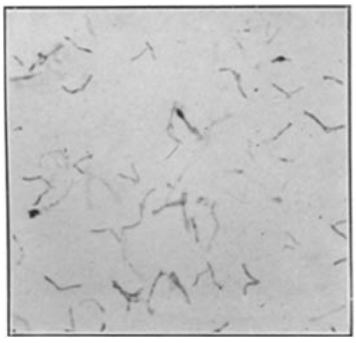


FIG. 10.



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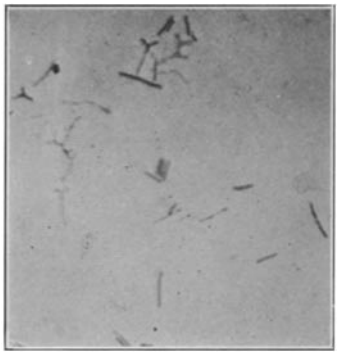
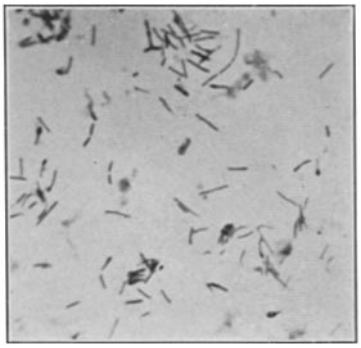


FIG. 12.

FIG. 13.



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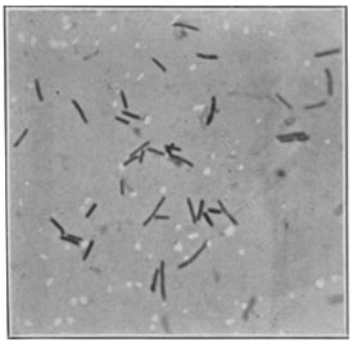

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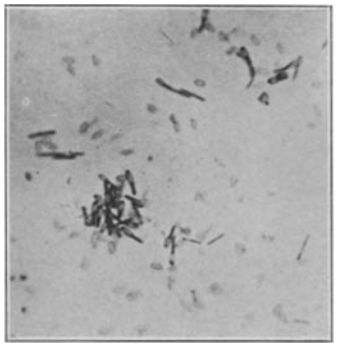

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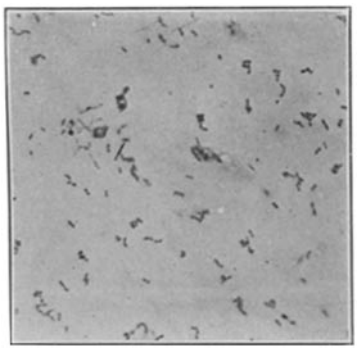

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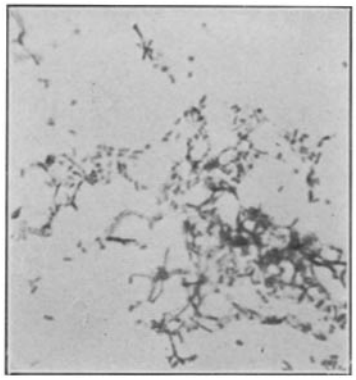

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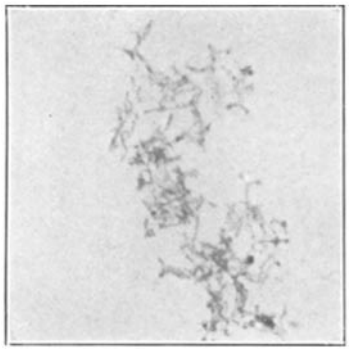

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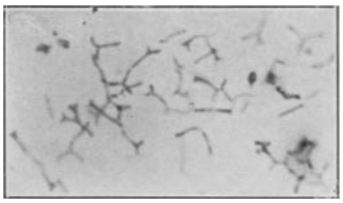
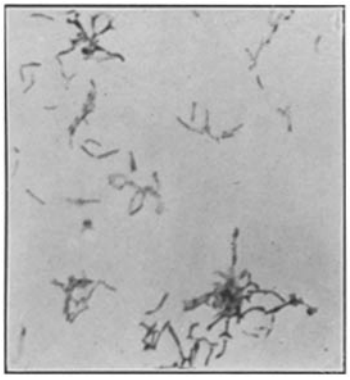


FIG. 20.



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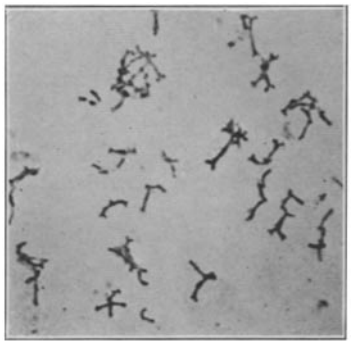

FIG. 22.

FIG. 12. Transition of bifurcating forms to straight type. From old modified Marino plates.

PLATE XII.

FIG. 13. Transition of bifurcating forms to straight type. From old modified Marino plates.

FIG. 14. Immediate semi-aerobic generation of *B. bifidus* just sprung from the anaerobic phase and showing transitory forms from non-branching anaerobic to straight sporulating phase. From a 24-hour colony in the deep layer of lactose agar near the anaerobic sphere.

FIG. 15. Semi-aerobic phase of *B. bifidus.* From a 2-day-old surface colony on glucose agar plate.

FIG. 16. Sporulation of semi-aerobic phase of *B. bifidus.* From a 3-day-old agar plate colony grown aerobically.

FIG. 17. Reversion of aerobiosis into anaerobiosis. Coccic stage with a few minute, bifurcated forms. From a semi-anaerobic cultivation of aerobic phase in glucose bouillon for 6 days at 37° C.

FIG. 18. Reversion. A step nearer anaerobiosis than the foregoing coccic stage. Extremely pleomorphic.

PLATE XIII.

FIG. 19. Reversion almost completed. From the third generation in reversing cultivation. Six days in glucose bouillon.

FIG. 20. Reversion completed. From dextrin serum water culture. Anaerobically cultivated.

FIG. 21. Reversion completed. Still numerous irregular types.

FIG. 22. Reversion completed. Very regular *B. bifidus* forms. From a lactose bouillon culture. Third anaerobic generation of the aerobic strain in glucose bouillon for 6 days at 37° C.