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BY

FILANDRO VICENTINI, M.D.

TRANSLATED BY

REV. E. J. STUTTER AND PROFESSOR E. SAIEGHI,

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# *ERRATA.*

—o—

Page 4, line 11, *for* "these" *read* "true."

Page 5, line 8, *for* "pus, corpuscles" *read* "pus corpuscles."

Page 8, line 3 from bottom, *for* "micrococci" *read* "pneumococci."

Page 10, line 4, *for* "per hour" *read* "in twenty-four hours."

Page 10, last line, *omit* "p. 1882."

Page 11, line 23, *for* "composition" *read* "decomposition."

Page 14, line 8, *for* "*k* and *k*" *read* "*k* and *k'*."

Page 20, line 18, *for* "a mixture of nitric acid and alcohol (1 : 3)," *read* "a mixture of nitric acid and water (1 : 3), and, soon after, absolute alcohol."

Page 21, line 2, *for* "in *g*" *read* "in *q*."

Page 66, line 12, *for* "these" *read* "there."

Page 95, line 27, *for* "hand" *read* "handle."

Page 109, line 35, *for* "which, although old, is not ours," *read* "which is not ours, but old."

Page 117, line 3, *for* "*x* and *x*" *read* "*x* and *x'*."

Page 132, line 1, *for* "saccharine" *read* "saccharose."

# PREFACE.

IT will be readily admitted by all that great interest centres about the study of the micro-organisms of the human mouth.

To the botanist, bacteriologist, and pathologist, no less than to the dental surgeon, the bacteria of the oral cavity offer a fertile field of investigation and present many questions, the solution of which has thus far been attempted in vain.

Up to the present, our investigations have been most successful in the direction of the pathological action of the mouth-bacteria, while, on account of the great variety of micro-organisms found in the mouth, and on account of the fact that many of them have resisted all attempts at cultivation on artificial media, no satisfactory classification of them has as yet been made or even attempted.

Consequently, any communications or investigations which may increase our knowledge of the flora of the human mouth, and help to bring about a classification which may serve as a basis for future work, or even any communication which serves to excite a discussion of the question, is deserving of thorough attention on the part of dentists, physicians, and natural scientists.

In this sense I recommend the Memoirs of Dr. Vicentini for a careful study. It is not the place here to enter into a critical examination of the views of the author; I refer the reader, in this connection, to the paper by J. Howard Mummery, of London, published in the *Transactions of the Odontological Society of Great Britain*, Vol. XXVI., No. 3, January, 1894. Suffice it to say that the deductions of Dr. Vicentini, if confirmed, would bring about a complete revolution of the views at present adopted by bacteriologists, resulting from the immense amount of work which has been done in this field for the last ten or fifteen years.

In order that my own position may not be misunderstood, I should add that, while attaching proper importance to the valuable work done by Dr. Vicentini, I am somewhat sceptical as to the correctness of his deductions. In the summing up of the author's work, which he has placed at my disposal in the English and French languages, I have found nothing which in my opinion justifies the conclusion that not only all bacteria found in the human mouth, but that even the pneumococcus, bacillus tuberculosis, and gonococcus are simply derivations of the same normal organism. If we go *so* far, we may with equal show of reason include the bacilli of diphtheria, typhus, cholera, etc., in fact, derive all known species of bacteria from one common parent plant.

In regard to the actual microscopical observations of Dr. Vicentini, Mummery *(l.c.)* reports having in one case found appearances very similar to those which Dr. Vicentini compares to grape-bunches. Personally, I have had no great difficulty in finding masses of granular bodies (micrococci) sometimes apparently accumulated about one central thread, sometimes about a mass of threads. Very often these masses are shapeless; in other cases they are shaped like a club or boomerang (Mummery), or bear a certain similarity to a bunch of grapes. As yet, I have not been able to devote much time to the study of these bodies, and can therefore offer no explanation whatever as to their signification.

In consideration of the importance of the question at issue, it is to be hoped that the work of Dr. Vicentini will receive a careful perusal, and that the investigations incited by it may bring about a better understanding of the biology of the micro-organisms of the human mouth than we have as yet been able to obtain.

W. D. MILLER.

*Berlin*; 1896.

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*FIRST MEMOIR.*

## ON THE SPUTA OF WHOOPING-COUGH.

Plate I.

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### A.

On the presence of Myelin in the Sputa of Whooping-cough, and on the Bacteria and Microphytes at times found therein, with remarks on the Bacteria of Sputa in general, and hints as to the Staining and Preparing of Specimens for the microscope.

SUMMARY:

§ 1.—Previous researches on the sputa of Whooping-cough (Cerasi, Poulet, Jansen, Ransome, Letzerich, Henke, Tschamer, Bürger, Moncorvo, Barlow, Michael, Afanasieff).

§ 2.—Notes on the Clinical cases, in which the sputa have been examined. FIRST CASE (*Sputa of May* 10, *May* 12, *June* 5; *sputum in Pneumonia*, *July* 31). SECOND CASE; THIRD; FOURTH; FIFTH, SIXTH; SEVENTH; EIGHTH; NINTH; AND OTHER CASES.

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§ I.

## PREVIOUS RESEARCHES ON THE SPUTA OF WHOOPING-COUGH.

THE epidemic of Whooping-cough which broke out in the town of Chieti, from April till July of the year 1888, afforded me an opportunity of making some few researches on the nature of the sputa in Whooping-cough. Feeling that the results of such investigations may shed some light on questions regarding the pathogenesis, phases, and peculiarities of disease, in so many respects still obscure, I venture to offer a short account of them in this memoir.

It will be well, first of all, to point out briefly what has been done in this matter since 1867 up to the present time.

The first account of the microscopical examination of Sputa in Whooping-cough appears in the monograph of Hagenbach, given in Gerhardt's treatise on the *Diseases of Children* and in the papers by Barlow* on the *Nature of Whooping-cough*, which appeared in four numbers of the *Lancet* in 1886.

* W. H. Barlow *On the Nature of Whooping-cough*, *Lancet*, 1886, Vol. I., pp. 870, 915, 966, and 1241.

In both these works mention is made of the researches of Poulet, in 1867, who, whilst examining the breath of infants, found a form of *Monas termo* or *Bacterium termo*, as well as another of *Monas punctum*, *Bodo punctum*, or *Bacterium bacillus*, which he thought to be the origin of the disease In the second and fourth sections I shall refer again to this.*

In the year 1868, Jansen, in his investigations, found bacteria in the sputum of Whooping-cough, but they were not identical with those described by Poulet, and he remained in doubt whether they were specific indications of Whooping-cough or simply microbes of the mouth.†

In the year 1870, Ransome ‡ brought before the Manchester Literary and Philosophical Society the first notice of the presence of a *Conferva* similar to *Penicillium glaucum* in the breath of subjects in Whooping-cough. His researches were corroborated, as regards measles, by those of Braidwood and Vacher. ||

These investigations were followed in 1873–74 by those of Letzerich, which are spoken of by almost all later writers. §

In the work of Cornil and Babes on Bacteriology, we find on this subject only the following short notice :—" He [Letzerich] has endeavoured to find and to cultivate the microbes in the sputa of Whooping-cough ; and has described and figured some enormous micrococci, which dispose themselves in irregular chains and in zooglæa. He even professes to have produced a convulsive cough

* Poulet, *Comptes-rendus de l'Acad. des Sciences*, 1867, LXV., p. 254 ; *Presse Médicale Belge*, 1867, No. 51. In the original Italian edition I overlooked the fact that in 1867 Dr. Cerasi, before the investigations of Poulet were made public, found in the sputa of Whooping-cough, under a power of 150 diameters, certain forms of fungi, similar to those described later by Letzerich, and which he called "*Oidium pertussis*." Nevertheless, these undoubtedly anterior researches escaped the notice of authors, who turned their attention to the sputa of Whooping-cough.—Cerasi, *Giornale delle Scienze Mediche di Venezia, Serie III., Tomo XII.* (*F. V.*)

† Gerhardt, *Tratt. completo delle Malattie dei Bambini*, *Versione Ital.*, *Napoli*, 1883, Vol. II., p. 474.

‡ A. Ransome, *Journ. Anat. and Physiology*, 1870, IV., p. 217 ; also *Proc. Lit. and Phil. Soc.*, Manchester, 1870, Vol. IX. p. 106.

|| Braidwood & Vacher, *Researches on Measles*, *Trans. Path. Soc.*, London, 1878, XXIX., p. 422.

§ Virchow's *Archiv.*, 1873, Vol. LVII., p. 518, and 1874, Vol. LX., p 409.

in rabbits by inoculating them, by tracheotomy, with micrococci. This work of Letzerich is absolutely contradicted by a communication from Bürger,* who finds in the sputa of Whooping-cough many small, rod-like, ellipsoid bodies, with a restriction in the middle. The sputa, to the naked eye, resembles tufts of matter, whitish at first, afterwards turning slightly yellow. It is these tufts, spread upon the glass slides, dried, stained with methyl violet, and bleached by alcohol, that show the rod-like bodies. They are found in or on the *cellules*, chain-like bodies being entirely absent. Bürger thinks that the drawings of Letzerich do not represent these parasites. According to Bürger, these rod-like bodies, so numerous in Whooping-cough, bear an intimate relation to the disease and its stages. He never cultivated them. As these micro-organisms can so easily be accidentally present both in the mouth and pharynx, they must always be looked upon with suspicion." †

Hagenbach gives a more precise account of the work of Letzerich in the monograph cited above. On the subject of the sputa in Whooping-cough, he quotes :—" During the period of convulsions we find in the mucus—fungoid filaments—sometimes in very great quantities, interwoven and ramified, and in which a vigorous formation of spores takes place.

" This fungus, a specific sign of the disease, can be detected with the naked eye. It is not reproductive in the mucus membrane, but lodged in the corpuscles of the mucus, and not on the epithelial cells. The course, duration, as well as the intensity of the paroxysms of the cough, vary with the fructification or propagation of this fungus, producing spasmodic irritation in the mucus membrane. The introduction of these fungi into the larynx or tracheæ of rabbits will in six to eight days bring about spasms of convulsive coughing. As a rule, the fungus of Whooping-cough invests the folds and cryptæ of the epiglottis, the larynx, and the tracheæ, finding its way even into the air-cells of the lungs, giving rise in its growth to catarrhal and inflammatory symptoms." ‡

* *Berliner klinische Wochenschrift*, No. 1, 1883.

† Cornil et Babes, *Les Bactéries et leur rôle dans l'Anat. et l'Hist. Path. des Malad. Infec.* Paris, 1885, pp. 556, 557.

‡ Gerhardt, *loc. cit.*, p. 474.

Further on :—" Letzerich has found the smaller bronchi of the inoculated rabbits, and even the air-cells, blocked by them, mixed with micrococci and a slender mycelium." *

In the fifth section relating to the fungi, which I have found in some specimens of sputa and their fructification, we shall have an opportunity of again referring to this.

Following the investigations of Letzerich, Henke in the same year (1874) discovered in the sputa, pus, corpuscles, and other round bodies (? young epithelial cells), full of minute particles in most lively motion, and which could be paralysed by solutions of quinine. † To myself, it seems very probable that this motion was not at all due to the corpuscles themselves, but was such a vibratory motion as is seen in all living cells, whether of the saliva, or white corpuscles of the blood, pus, mucus, or young epithelium cells, wherever an osmosis takes place. Very striking examples of such motion is seen in the corpuscles of the saliva, of mucus, of normal sputa, of nasal mucus, or, better still, of the mucus of the urethra, by allowing water, pure or coloured with aniline, to run into the fresh preparations.

Tschamer, of Gratz, in his many researches during the year 1870, had not observed, except in a very few cases, any microbes in the sputa of Whooping-cough ; *and then only such as are found in healthy sputa, especially that expectorated in the morning.* But in 1874, one or two days before the convulsions set in, he came across a reticulated mycelium, and many round or ovoid spores of different sizes. These were at first colourless, but subsequently became yellowish or of a darkish red colour. He thought these to be very similar to, if not identical with, the dark mould on decaying orange or lemon peel, and looked upon the spores of this mould as the germs, or the pathogenic cause, of Whooping-cough.

The observations of Tschamer were confirmed by Oltramare and others, but contradicted by those of Rossbach of Würtzburg. But more of this in our fifth section. ‡

In the year 1883, Bürger of Bonn describes, as we have seen

* *Ibidem*, p. 485.

† *Arch. für Klinische Medicin*, XII., 1874, p. 630.

‡ Barlow, *loc. cit.*, p. 916.

in the extract from Cornil and Babes, the microbes in the sputa of Whooping-cough as sometimes linking themselves in chain-like bodies, the larger ones being constricted in the middle and others forming clusters in colonies. He took them to be quite distinct from those of the *Leptothrix*.*

In the same year (1883) Moncorvo, of Rio de Janeiro, observed swarms of micrococci scattered about the mucus or inclosed in the pus corpuscles or in the epithelium cells, which seemed to him to gradually decrease or disappear altogether, as the disease ran out its course. Local applications of Resorcina paralysed the movements of these micrococci.†

Full particulars of these researches from those of Tschamer to those of Moncorvo will be found in Barlow's papers.

In the year 1886, at the end of his investigations, Barlow published the results of his microscopical examinations of the sputa in Whooping-cough—examinations undertaken with the help of Broadbent. A 1/12th inch objective was used, magnifying to 800 diameters. The preparations were stained with methyl violet and showed numbers of nuclei of pus corpuscles, fibrinous particles, and a few epithelium cells, some covered with minute micrococci in chain-like lengths or masses (zooglæa). Though similar micrococci were to be seen all through the rest of the field, nevertheless it was evident that the principal nidus was in the epithelium cells. Of this remarkable fact I shall have more to say. From these observations Barlow was led to believe that the cause of Whooping-cough was to be found in the disquamation of the epithelium cells of the larynx, brought about by foreign growths.‡

Michael of Hamburg, in the same year 1886, declared his firm opinion to be that Whooping-cough arose from a reflex and spasmodic action, partially of the pneumogastric nerves, but principally from that of the muscles of the upper part of the larynx, caused by a specific irritation of the nasal mucus membrane, produced by certain microbes. On this account he recommended nasal

* *Berliner Klinische Wochenschrift*, 1883, No. 1.

† Moncorvo, *De la Nature de la Coqueluche et de son traitement par la Resorcine*. Rio de Janeiro, 1883. *Archiv. di Patalogia Infantile*. Napoli, '85.

‡ Barlow, *loc. cit.*, p. 967.

insufflation of gum benzoin, quinine, and nitrate of silver mixed with calcined magnesia.*

Hack, Schadewald, Wille, and Sonnenberger took the same view, as may be seen from an article in the above-mentioned periodical,† and from a short notice of it by Morgagni in the same year.‡

But these writers do not speak of the morphology or biology of these nasal parasites, nor of the methods of staining them for observation. Nor is it clear that their opinions are derived from their own bacterioscopical examinations, or are simply hypotheses put forward to uphold particular therapeutical theories, or to explain the true or supposed virtue of certain local remedies as those above mentioned, or others, such as iodiform, salycilic acid, boracic acid, injections or inhalation of corrosive sublimate, tincture of iodine, and alum, || or the use of other local or general applications of quinine, antipyrin, etc.§

Until more is known of this parasite, I think it best to leave its discussion, turning my attention more exclusively to the examination of the sputa in Whooping-cough. My conclusions, as will appear, are not opposed to the idea of nervous spasmodic action, and are compatible with other researches as to the seat of its first origin.

The following notice in the *Lancet* of December, 1887, will show results of the work of Afanasieff:—

"Doctor Afanasieff has succeeded in finding and cultivating what he believes to be the true bacillus of Whooping-cough. This microbe differs distinctly from all other bacteria which have been described. It is somewhat like Friedländer's Pneumonia bacillus, but is shorter and thinner than the latter; besides, in gelatine it

* Michael, *Deutsche Medicinische Wochenschrift*, 1886, No. 5, p. 74.

† Sonnenberger, same article. 1887, No. 73.

‡ *Morgagni*, 1888, Part II., No. 20, p. 256, and No. 39, p. 473.

|| *Vide*, among others, *Nasal Treatment of Whooping-cough*, *Lancet*, 1887, Vol. I., p. 136.

§ As, for example, *Deutsche Medecinische Wochenschrift* and *Morgagni* in articles cited above.

does not form nail-shaped cultures, those which are produced having no hemispherical head.*

"Its potato cultures too, are quite different from those obtained from Friedländer's bacillus. Afanasieff's bacillus exhibits a remarkable degree of vitality, for jelly cultures that have become dry and have been kept for four months, appearing under the microscope to be more or less destroyed, are still capable of producing fresh cultures when fresh media are inoculated from the dried mass.

"Dr. Afanasieff's researches were chiefly made from the sputum of some of his own children who were affected with Whooping-cough. The mouth was well washed out with a permanganate solution, and the mucus coughed up after the next paroxysm or two examined. In this mucus, after staining with methyl-violet, and in the pus-corpuscles contained in it, the bacilli could be seen with a magnifying power of from 700 to 1,000 (Zeiss's eye-piece 3 or 4, 1/12 inch oil immersion objective) as short rods, sometimes single, sometimes in twos, or even in short chains running in the direction of the mucus, sometimes again in small cultures. Their length was from 0·6 $\mu$ to 2·2 $\mu$. Of course other bacteria were found. Pure cultures, however, were easily made on agar-agar, meat peptone, jelly, potato, etc. Dogs and rabbits were inoculated with a fluid culture, mixed chloride of sodium solution, some by means of injections into the trachea, others by direct injections into the lungs. All the animals were seriously affected, and many of them died. The symptoms were somewhat similar to those of Whooping-cough, including cough, dyspnœa, and redness of the eyes. Many of the cases were complicated by broncho-pneumonia. On examining the bodies of those animals which died, the mucus membrane of the air passages were found much reddened, and coated with a tenacious clear mucus, in which, as well as in the pneumonic patches in the lungs, the bacilli were found. Similar appearances and the same bacilli were observed in the bodies of children who had died from Whooping-cough. As to treatment, inhalations, and spray of various antiseptic drugs would appear

* Dr. Afanasieff has personally examined and cultivated the so called micrococci (*Comptes Rendus hebd. Société de Biologie*, May 21, 1884, p. 356). As to the club-headed cultures, see among others Cornil and Babes, *loc. cit.*, Pl. XXVII., Fig. 27 (F. V.)

to afford the most ground for hope in relieving and shortening this complaint. The author points out that the quinine, benzoin, and other substances which have been used as applications to the usual mucus membrane by Michael and others, under the idea of combating a reflex affection, are really, perhaps, beneficial from their property of destroying bacilli." *

The *Medical Times* of Philadelphia, of April, 1888, gives a shorter notice of Afanasieff's discoveries.†

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§ II.

## A SHORT ACCOUNT OF CASES, IN WHICH THE SPUTA HAVE BEEN EXAMINED.

The thought of examining the Sputa in cases of Whooping-cough did not occur to me till the epidemic of this year, when I came across a truly typical case of Whooping-case, typical both in its clinical symptoms and in the character of the Sputa. The principal features I have found in Sputa of Whooping-cough, as will be seen, were the presence of *an extraordinary and peculiar abundance of masses, particles, filaments, and granules of myelin*, and it was through observing the quantity of myelin that caused my surprise in this first case, a quantity that took up quite one half of the surface of the preparations examined. Perhaps if I had not come across such a striking case, I should not have given so much importance to the presence of myelin nor renewed my researches in other cases. The microscopical examinations were undertaken on this occasion through a special interest in the little sufferer and the fear of some other threatening complication.

### FIRST CASE.

A.S., a boy of 8 years, plethoric, robust, and well developed for his age, was in the latter part of March seized by attack of measles with a distinct nasal catarrh, confluent eruption, somewhat hæmorrhagic. After the disquamation, he showed symptoms of

* *The bacillus of Whooping-cough.—Lancet*, Dec. 3, 1887.—Vol. ii, p. 1131.

† *Philadelphia Medical Times. April 2, 1888.*—No. *530., p. 403.*

bronchial catarrh, which, though slight at first, became more distressing by the middle of April, and at last took the form of Whooping-cough.

The boy was subject to some twelve paroxysms per hour, with facial adema, ecchymosis of conjunctive membrane, epistaxis,* and a severe form of *quintes*, as the French call them. He had no signs of capillary bronchitis or pneumonia, nor any fever. The case was therefore truly typical in the form of the paroxysms and in the absence of other complications.

*Sputa of May 10.—(a)* The first sputum taken for examination was after the third week of the period of convulsions of the Whooping-cough; it was colourless. Hyaline, very viscid, and found to be free from any residuary particles of food. It contained but few pus corpuscles, the whole mass being composed almost entirely of the elements which will be described. No red blood-corpuscles were found. A fair quantity of buccal epithelium cells and pavement epithelium cells from the air-passages. I thought it would be superfluous to reproduce these in the plates, with the exception of a small particle of buccal epithelium cell, as in *f*, Fig. 2. In Fig. 1, *h* is a salivary corpuscle, stained with gentian violet, magnified to 1,750 diameters. Vibratile epithelium cells from the air-passages were to be seen; a small one is shown in *f*, Fig. 1, coloured in the same way and magnified to 2,500 diameters. Rather a moderate number of ellipsoidal cells of epithelium were present from the air-passages, called sometimes alveolar epithelium cells. This is not the place to discuss whether or no these cells truly come from the air-passages or from the air-cells.† One thing is certain—that they are found often covered with pigment grains and with myelin in sputa of all kinds. I have found them in those of simple hoarseness.

In Fig. 1, *g*, one of these cells will be seen, in its natural state, with the granules of myelin magnified to 400 diameters.

From the very first, I was greatly surprised to find that nearly

* Epistaxis—bleeding from the nose.

† Senator held them to be epithelium cells from lower parts of the bronchial mucus membrane. (Bizzozero, *Man. di Micr. Clinica*, Milano, 1882, p. 1882, pp. 144—145.)

the half of the surface of the preparations consisted of numerous masses of myelin, in all sizes and shapes. Fig. 1, *d*, shows one rather smaller than others, drawn in its natural state, magnified to 400 diameters. Among these masses were seen myriads of particles of myelin, some isolated, some grouped together, as shown in *a*, *a*, *a*, *a*; others are shown in *b*, *b*, *b*, *b*, *b* (stained with gentian violet), both magnified to 1,000 diameters. Besides these, there were numberless grains of myelin smaller than the most minute bacteria and barely visible, unstained, but rendered quite distinct by gentian violet. These are shown in *b* on the left of the grouped particles, and magnified to 1,000 diameters, but better still in Fig. 2, *t*, where they are magnified to 2,500 diameters. Some portions and detached filament are shown in Fig. 1, *b* (1,000 diam.), and at *i* are shown, for comparison, some minute particles and some of the smaller granules unstained, as given by Dr. Beale, magnified 2,800 times.*

It was evident that the viscosity and hyaline character of the sputa was due to the great amount of myelin present, but of this more later on, as well as of the bacteria and bacilli found in the sputa. It will be enough now to say that both in the sputa of this case and subsequent ones, there were found, even when fresh, many forms of spirilli, *Leptothrix buccalis*, *Vibrio*, *Bacillus subtilis*, which increase day by day with the composition of the mass. Too many plates and too much space would be required to describe singly all the forms and varieties of the bacteria and bacilli. Therefore, only the principal forms will be given in the illustrations.

The different bacteria and bacilli of Whooping-cough are stained with gentian violet. Fig. 2 shows a group of minute bacteria in *a* (800 diam.), some of which are reproduced in *b* (2,500 diam.). In *c* will be seen two chain-like bacteria—one shorter and twisted, the other longer (800 diam.); *d* shows a part of the longer chain, magnified to 2,500 diameters. Two dumb-bell bacteria are shown in *e* (2,500 diameters), one of which is quite capsular in appearance. Of such dumb-bell bacteria there was great abundance, some spread throughout the mucus mass, but most of

* Beale, *The Microscope in Medicine*, London, 1878, Pl. XXI., Fig. 6.

them swarming around buccal epithelium cells, specially around those showing signs of disintegration or corrosion. In *f* will be seen a portion of buccal epithelium cell invaded by both these forms of bacteria under the power of 2,500 diameters.

Such dumb-bell bacteria formed the greater part of the bacteria of the sputum, seemingly identical with those discovered by Poulet in vitiated air and by Bürger in sputa.

Some extremely minute bacteria and bacilli are shown in *g* (2,500 diameters), with two specimens of curved diplococci morphologically identical with the so-called *gonococci* of Neisser; but these curved diplococci were only found in some few portions.

In *h* is reproduced (2,500 diameters) a corpuscle shaped after the *pneumococcus* of Friedländer, but larger, and which might have been a very small pus corpuscle misshapened, as shall be mentioned later.

In the first examination but few specimens of the forms *i*, *k*, and *s* were observed. Few also of those interwoven, as in *u* and *u*, were seen. These last appeared afterwards in greater numbers in this same sputa and also in other sputa.

Divers kinds of bacilli also were noticed. Two are shown in *m*, *m* (2,500 diameters), which seem to be more mature forms of the young bacilli, seen in *n* and *n*, which were found in other cases.

These examinations of bacteria and bacilli were made with a homogeneous immersion lens, 1/18th inch, and a Dujardin condenser of the improved pattern, made by Messrs. Bezu, Hausser, and Co., successors to Hartnack and Prazmowski. The drawings have been faithfully made from the preparations.

(*b.*) The day after, May 11, other portions of the same sputum were examined and found to contain the above forms of microbes, in increased numbers, specially the beaded strings, as in *i* and *k*. The forms shown in *l* (2,500 diam.) were observed, of which some may represent smaller specimens of the forms *d* and *e*, others are bacteria, very small and globular or oval in germination. From these oval forms may be derived those of a long biscuit shape; of these the greater part were capsular in appearance. They are seen low down and on the left in the figure, some in twos, some in chain-like lengths, generally grouped round the buccal epithelium cells or penetrating the cells themselves.

Other bacteria of this group are, instead, representatives of the same forms *d* and *e*, increased in size, with their heads not yet united. Many individuals have a capsular appearance as seen on upper part and to the right of the figure, at *l*. There seems to me every reason to believe the diplococci, though smaller, to be similar to the pneumococcus of Friedländer, of which Dr. Afanasieff gives an account. It may be here observed that I have found analogous or identical forms in sputa under other circumstances, in normal sputa and in sputa during affections of the air passages, likewise in saliva, nasal mucus, urine, and even in spermatic fluid.

On the left and above the figure being described there is a curved diplococcus, similar to that in *g*, but somewhat larger ; in the chain (*p*, *p*, Fig. 2) there are some still larger, and the similarity naturally suggests that the drawings *g*, *l*, *p*, are but three degrees of development and increase of one and the same bacterium.

It was, perhaps, by inadvertence, that no trace of fungi was noticed the day before, but on the eleventh I observed germinating filaments of fungi of fair size. They were, at first, all after the same type, as in Fig. 3, *d*, where they are shown stained with gentian violet and magnified to 800 diameters. It should be said that while the figure shows them with a regular elliptical outline, as in *e*, *f*, *g*, etc., they were in reality somewhat flattened at the ends.

Not to anticipate the description, given later on, of this fungus, I would merely state that these filaments became more conspicuous during the following days, and a great many *vescicular gemmules, sacculi, or sporidia* were noticed, some yet attached to the filaments, others already fallen off. As these closely resemble those spoken of by Hagenbach as observed by Letzerich used in his inoculation, they are given in Fig. 3, *b*, *b*, stained with gentian violet, and in *c*, with carmine [Fig. 3*c* is printed in violet, to be uniform with Fig. 3 *b b*], magnified to 2,500 diameters.

(*c.*) During the following days there appeared in the sputa (which had been kept in a tightly covered porcelain vessel), among the larger filaments described above, other smaller ones, with many small rounded spores of a light greenish colour.

In the same interval the larger filaments had increased in numbers to such a point that by the 16th (the sputum was then five days old) one could count some twenty or thirty filaments both small and large in one single preparation. It was probably due to the multiplication of the greenish spores that the mass of the sputum took the same greenish colour and gave off a musty smell, which increased till the whole dried up in about a month at a temperature of 20° or 22° C.

It was during this time that I observed the fructification of both these fungi. That of the larger ones, as the filament, *d*, is shown in Fig. 3 *k* and *k* (140 diameters); that of the smaller ones, the filaments of which are not shown, is given in *n. o. p.* (800 diameters). Spores detached in the fructification of the first fungus will be seen in *l* and *l*, those of the second in *m* and *m* (1,000 diameters).

I intend to refer later on to my observations on sputa after boiling and on the dust deposited in the room of the sick boy.

These few notes will suffice for the present in regard to the sputum of May 10.

*Sputum of May* 12.--On this day another portion of the sputum was taken and it gave the same results as that of the day before, showing the same extraordinary amount of myelin. In these were present in great quantities the chain-like or beaded bodies budding at one extremity as in Fig. 2. *i*, or at both ends as in *k*. These beaded forms have been found in other cases of Whooping-cough, in other sputa, in saliva and various other substances.

On the second day large numbers of the filaments of fungi appeared in this sputum, as before described. I have not had the leisure to follow the development of the filaments, nor to ascertain whether the smaller ones made their appearance.

*Sputum of June 5*.—It was during the seventh week of the convulsions that an attempt was made to ascertain if the same amount of myelin was still to be found, and it was observed in the same large quantity. No great notice was taken of the bacteria in this sputum, but the large filaments of fungi and their germination were noticed, as in the preceding cases.

Towards the end of June, the cough became less intense and less frequent, and by the end of this month might be said to have passed away; but the boy still brought up from time to time a yellowish mucus, such as is seen in a case of very mild catarrh.

But the clinical history of the case does not end here. The boy on the 28th of July, through his profuse perspiring, was seized with an attack of tonsillitis, and was slightly feverish. Two days afterwards, on the 30th, at 10 a.m., he was in a high fever, subject to shivering fits, with heavy breathing and a sharp pain on his right side. By the evening his pulse was 160 and his temperature 40·1° C. During the night some of the expectorations were tinged with red. In the following morning (the 31st July) a fresh portion of sputum was taken for examination under the microscope.

*Pneumonitic Sputum of July 31st.*—This sputum was somewhat tinged with red, and on being examined was found to contain a large number of red blood-corpuscles, a fair quantity of ellipsoidal epithelium cells, covered with small granules of myelin; throughout the mass some few smaller particles of myelin were observed, which seemed to have fallen away from the ellipsoidal epithelium cells. But no vestige was to be seen of the innumerable particles nor of the numerous filaments and masses of myelin which had been found in such large quantities before in the sputa during the Whooping-cough.

What had become of the extraordinary abundance of myelin? If the copious formation and elimination of this matter arose from some special condition or idiosyncrasy of the subject, there would be no reason why they should not appear in this lung attack; all the more, as the quantity of sputum from the lungs in twenty-four hours was very moderate. So that an equal amount of myelin in this sputum would have given a much higher per centage than that shown during the Whooping-cough. But this matter will be taken up later.

The bacteria found in this sputum were not in any way different from those that are usually met with in other pulmonary attacks, and, on the whole, were not unlike the bacteria of sputum in cases of Whooping-cough. The forms *e*, *f*, and *f′* (Fig. 2), with a capsular appearance, were rather abundant. Many forms in couples and in chains were present; they are shown in *p*, *p*, and are met with in cases of pulmonary complaints. Many of them had a capsulated appearance, sometimes enclosing two in the same transparent envelope, as in *p′*, or in threes and fours, and in lengths of six or more. All the forms *e*, *f*, and *f′*, as well as the forms *p*, *p*, and *p'*, were seen either covering or penetrating the buccal epithelium cells.

It should be noticed that the larger bacteria, as in *p* and *p'*, are found—and in great quantities, too, at times—in the sputa of whooping and other sputa. But the author never met with the forms curved after the fashion of the so-called *gonococci* intermixed in the chains except in Whooping-cough; nor observed these forms having in Whooping-cough a capsular appearance.

Besides the above, the forms shown in *l'*, to the right, and in *l* under the figure were plentiful; forms which, together with that in *f'*, may be classed with so-called *pneumococci*. They are certainly numerous in the sputa in cases of pneumonia and pleurisy cases, and are to be found generally in other sputa and other substances.

It was very easily noticed in this specimen of the sputum how the forms *l'* and *f'*, with the knob-heads separated, passed into those at *l'* with the knobs as yet only partly joined, and into those at *e* and *f* all in one, and all the more, as these last two forms are the same as those with the knobs separated, only seen foreshortened.

Along the striæ of the mucus were seen swarms of very minute bacteria; but they were similar in form to those shown in *f* and *p*, and may be thought to be only younger specimens of the same type.

During the following day—August 1st—a greatly increased number of these minute bacteria were found in other preparations of the same sputum, and others more minute were observed in such beaded shapes, as shown in Fig. 2, *s*, and in interwoven masses, as in *u* and *u*. The first are magnified to 2,500 diameters; the second, for the sake of space, only to 600 diameters. These forms are found in other sputa and will be further described. Among the swarms of the minute bacteria, I observed some slender bacilli in their first growth (see *b'*, 2,500 diam.), where will also be seen some very small bodies, perhaps micrococci, which appear to be in process of being cemented together by a finely granular matter forming between them. The chain-like bodies, as in *c*, were present, and more than ever the forms *p* and *p'*, which appears still more evidently to force their way into the buccal epithelium cells.

The forms shown in $b'$, $f'$, $n'$, $p'$, $l'$ on the left, $l$, $l'$, lower down, and in $x'$ are stained with methyl violet, and are from cases of inflammation of the lungs or pleurisy; it is my experience that methyl violet in these cases renders the capsular appearance more distinct.

I shall have more to say on the sputa of Pneumonia or Pleurisy; as regards the boy the disease soon ran its course, with a morning temperature of 39°C., 39·4°C., and evening temperature of about 40° C. The fever abated on the fifth and the boy was well again by the twelfth of August.

Naturally the results of the examination of the sputa in the case of this boy led me to further researches in other cases of Whooping-cough. In this there was a certain difficulty, for in the case of infants the expectoration is usually very limited, only small portions can be obtained, and in these the very elements sought for may easily be wanting. For myelin is not always diffused to the same amount in sputa from different parts of the bronchial secretion. And then, again, in private and ordinary practice it is not always easy or becoming at times to seek for material for examinations or researches that do not tend to the immediate relief of the patient, and in fact I could only in fourteen other cases find means of examining the sputa. These cases will be considered in the following pages.

### Second Case.

On the 18th of May I examined the sputa of a little girl aged five years during the seventh week of the convulsions. The sputum was to all appearance a puriform mucus. Red blood-corpuscles were to be seen, but in no great quantity; a fair quantity of pus corpuscles; a few ellipsoidal epithelium cells were present, together with many buccal epithelium and pavement cells from the air-passages, but very few of the small vibratile cells from the same.

Very few particles of myelin were found here and there in the mass. However, the day after, the sputa having been kept in a closed tube, the myelin, being a lighter body, came to the surface in such quantity as to form about one-tenth of the whole quantity. This habit that myelin has of rising to the surface of sputa will be alluded to again.

The bacteria were much of the same forms as in the preceding case, but in far greater quantity, being found principally upon the buccal epithelium cells.

### Third Case.

The sputa of an infant of ten months was examined on the 19th of May, during the first week of the convulsions. Several red blood-corpuscles were observed, many pus corpuscles, very few ellipsoidal cells of epithelium, and fewer vibratile cells. Buccal and pavement cells of epithelium were present as before. Very few granules of myelin were found, and the bacteria were much the same as those observed in the first case. A good many specimens of the form *o*, Fig. 2 on the left ( × 2,500 diam.), presented themselves. This form, as can be seen from the figure, is much the same as that in *g* and *l*; in this case, there appears something of the nature of a cementing medium between the cocci. Some minute diplococci, with well-defined capsules feebly showing the darker cocci within, are represented in *o* (upper part of Fig. 2). There were other more minute bacteria, but of the same form as those before described. The infant died during an attack of convulsions.

### Fourth Case.

I was able on the 20th of May to examine the sputa of a boy two and a-half years old during the first week of the convulsions. The same morphological characters were found, and myelin was in the same small quantity. The bacteria were the same as those of the first case; but a number of bacilli appeared, some longer, some shorter, showing internally signs of segmentation, granular masses or bacteria, or mere specks of coloured matter (see *n*, Fig. 2, magnified 2,500 diameters). There will be occasion for dwelling on the similarity of this form and that in *m*, *m*, referred to in the first case, as well as on the probability of the origin of the larger bacillus, *n*, from the mass, *u*, *u*, and that of the smaller bacillus, shown close by, from that of the beaded form, *v*.

Among my notes there is no mention of fungi in these three last cases, but perhaps they may simply have been overlooked.

### Fifth Case.

On May 24 the sputa were examined in the case of an infant of five months during the third and fourth weeks of convulsions, in which the *quinte* was very pronounced. The sputum was dense and so thick, that in order to spread a portion on the slide it was necessary to dilute it with a drop of chloride of sodium solution. Red blood-corpuscles were absent, but a few pus-corpuscles. Many ellipsoidal epithelium cells, some few vibratile epithelium cells, with the usual pavement cells, were found. Myelin was in almost the same abundance as in the first case. The smaller particles and lighter portions could be observed to collect on the edges of the preparation, enclosing within the liquid belt the thicker and viscid material. The bacteria were very similar to those in the first case.

On the following day, the germinating filaments of fungi and the vesicular gemmules of the first case were again met with in another portion of the same sputum.

This infant recovered from the Whooping-cough, but died three months afterwards from inflammation of the right lung.

### Sixth Case.

The sputum of a boy four years old was examined on the 24th May during the fourth week of the convulsions. The attack was of a much milder form and the cough less frequent than in the preceding case. Here, too, the sputum was dense and thick, containing some few red blood-corpuscles, a few pus-corpuscles, scarcely any ellipsoidal epithelium cells, but about the same amount of the pavement and vibratile epithelium cells as in the case above. Myelin was present in a fairly abundant, although not in such an enormous quantity as seen before. The forms of bacteria, *p* and *p*, were observed, besides those described above. One bacillus was seen slightly coloured, enclosing some darker bodies, small masses—perhaps bacteria—irregularly disposed; it is shown in *n* on the right and magnified 2,500 diameters.

It appeared to me to be a younger specimen of the bacillus *m m*, met with before.

### Seventh Case.

(*a*) On May 25th I examined the sputum of a boy two years old during the second week of the convulsions. The cough was not so very distinct from that of an ordinary cold, but harsh, and taking into consideration that other boys of the family were evidently suffering from Whooping-cough, I considered it to be of a distinctly specific character. The boy had had a fit of convulsions two days before, following a severe attack of indigestion. In the stool of the lad portions of orange-peel were found. He was teething and feverish.

In the sputum examined red blood-corpuscles were abundant, as also pus-corpuscles and the ellipsoidal epithelium cells; but of myelin little or nothing could be seen.

(*b*) The sputa were again examined on the 5th of June during the fourth week of the attack, which was still of a rather dubious character. The fever had disappeared. I found a good number of particles and granules of myelin, many masses and many isolated specimens of the beaded bacteria (Fig. 2, *s*, magnified 2,500 diameters). These bacteria, in form, size, and disposition, were very similar to the so-called Koch's bacillus or tubercular bacillus, but in a dried preparation they were completely bleached by a mixture of nitric acid and alcohol (1 : 3). Many bacteria were observed similar to those in *p*, *p*, which, as well as those beaded forms, *s*, were principally crowding about or within the buccal epithelium cells, as has been before mentioned. Here, too, were observed the larger bacilli of the form, as in *m*, *m*.

## Eighth Case.

(*a*) On the 27th of May the sputum of a little girl aged seven was examined during the fifth or sixth weeks of convulsions. The girl had some slight and irregular attacks of fever, but every symptom of either capillary bronchitis or catarrhal pneumonia were altogether wanting. Red blood-corpuscles, pus-corpuscles, and ellipsoidal epithelium cells were present. Myelin was found in considerable quantity; the particles and free granules were so numerous in proportion to the few epithelium cells, that they could not be said to be simply a diffusion of the myelin so frequently found clothing such cells. Among other forms of bacteria, those shown in *p*, *p*, were in extraordinary numbers, some arranged in line, others united together in twos and fours. Of those in line with one another, some seemed bent and to have a striking likeness to *gonococci*, as in the figure drawn from this very preparation. In Fig. 2, *r*, is shown a large bacillus with four articulations, and in *g* a form with one articulation. Both were observed in this sputum (magnified 2,500 diameters).

(*b*) On the 9th of June, the eighth week of the convulsions, the sputum was again examined, but little or no myelin was found. In this sputum the bacteria in the largest numbers were the groups, isolated specimens, or beaded chains shown in *d*, *e*, and *f*.

## Ninth Case.

On the 13th of June I examined the sputa of a little girl six years of age, during the fourth week of the convulsions. Some few red blood corpuscles were found, a good many pus corpuscles, only a few ellipsoidal cells of epithelium, many vibratile epithelial cells, and others as before. Myelin in this sputa was so plentiful as to rival the abundance found in the first case. The bacteria were very much the same as in the other cases, but among them were observed some diplococci, apparently capsular, similar, though smaller, to those shown in *l* and *l'*. There were also some slender tufts of *leptothrix*. I have no record of fungi in these last four cases.

## Other Cases.

During the month of June I examined other sputa in six different cases of Whooping-cough, and in these I limited my research to the observation of myelin and fungi. Myelin was found always present in large quantities in severe cases and in the height of the attack, but in more diminished quantities in doubtful cases, and during the beginning and decline of the attack. The larger germinating filaments of fungi were met with in one case only.

Altogether in fifteen cases of Whooping-cough, nineteen examinations of the sputa were made, and the results may be briefly stated to have been :—

(1) That myelin is not found to show any relation with fever or inflammatory symptoms which may accompany an attack of Whooping-cough, nor to bear any proportion to the quantity of the ellipsoidal cells of epithelium ; but to show evident relation and proportion to *the intensity of the nervous excitement.*

(2) That the bacteria and the bacillii in Whooping-cough sputa are of a most varied character and such as are found in other sputa and in many other substances.

(3) That the large germinating filaments of fungi have been observed only in a minority of cases—in three cases out of fifteen, and in five specimens of sputa out of the nineteen examined; they were, therefore, undoubtedly absent in the majority of cases.

The following sections will be devoted to the general discussion of myelin, bacteria, and fungi in the sputum of Whooping-cough.

## § III.
## NOTES ON MYELIN.

### Myelin in general.

Dr. Beale, in his treatise, "The Microscope in Medicine," thus describes myelin:—"Myelin is a colourless, glistening, semi-fluid substance, prone to form drops, and capable of being drawn out into long threads, which curve and twist into the most peculiar forms. If the observer examines a portion of the white matter of the brain or spinal cord in water, he will recognise this substance without difficulty. The masses often exhibit double contours, and not infrequently many lines may be discerned equidistant from one another, but varying much in thickness and in intensity (Pl. XXI., Fig. 6*a*). It is present in the liver, and may be detected in almost all the tissues. In many tissues of the adult, myelin exists in considerable quantity in the outer part of the cells (Fig. 4), and in old age a still larger proportion is present. This substance was first described by Virchow, but Beneke has shown that it may be obtained from all the tissues of the body, and that it exists even in plants. It is soluble in hot alcohol, ether, and turpentine. Cholesterine is a necessary constituent of myelin, and can always be obtained from it. Iodine tinges myelin of a reddish-brown colour. If sulphuric acid be added, a blue or violet colour is induced. This reaction probably depends upon the presence of cholesterine. Beneke showed that myelin gave the reaction characteristic of the biliary acids upon the application of Pettenkofer's test." *

* Beale, *loc. cit.*, page 189.

The words *gocciole*, *goutteletles*, *drops*, *myelintröpfchen*, used by the English author and by Virchow and others,* do not seem to me to express so well the physical structure of myelin and its many forms as that more suitable one which Ranvier adopted, viz.—filamentous buttons. Ranvier, in his treatise on Histology, thus expresses himself on myelin in nerve-fibres :—

"When subjected to the action of water, the axis cylinder, being laid bare, swells until the myelin surrounding it is broken up into filamentous buttons. They may be described as transparent threads wound up upon themselves. These threads swell little by little, the outlines becoming less and less distinct till they seem to merge into each other, and n about half-an-hour or an hour the filamentous buttons arise, as little balls of various dimensions, showing a very refractive outline, the concentric striæ corresponding somewhat to the original threads. These masses of myelin take the most varied shapes, from that of a cylinder to that of a sphere, the *bizarré* forms in which they present themselves defying all description. Finally, myelin, when isolated, is entirely transformed into spherical or cylindrical masses of different lengths, showing a double contour, presenting a highly refractive border of varying width. This gradual transformation of myelin does not in the least resemble coagulation. On the contrary, all the fibres that at first are to be seen absorbing the water seem to swell, to run together, and so produce these rounded masses with the characteristic double outline.

It is only where the nervous fibres have been divided by sectioning or tearing that the myelin breaks away in the additional liquid and takes the various forms that we have already spoken of. The natural contour of the fibre remains regular, however, during the working of the preparation, unless, by chance, there is some other lesion from which other threads of myelin may flow, as from the part operated upon."†

It is true we owe to Virchow the first description and the very name of myelin.‡ Nevertheless, myelin had been discovered

* Jaksch, *Manuel de Diagnostic des Malad. Inter.*, *par les Methodes Bactériologiques*, etc. *Trad. de l'Allemand*, Paris, 1888, p. 63.

† Ranvier, *Traité Technique d'Histologie.* Paris, 1875—82 (ending '89), p. 720.

‡ Virchow, *Archiv*, 1854—6, p. 562.

before, for these masses, filaments, and drops which escape from the nerve-sheath are described in the middle of this century by Henle in his "General Anatomy." He speaks of them as the *globules of medullary substance, which coagulate.**

Under the word myelin in Selmi's *Chemical Dictionary*, we find the following remarks :—"Myelin may be obtained by treating the yolk of a boiled egg with alcohol and subsequent evaporation, or in like manner from the substance of the brain, crystalline humour, and from other tissues. Beneke has found it in the snail, *Helix pomatia*, in other lower animals, and in the buds, flowers, and seeds of plants, together with cholesterine. Liebreich thought it to be a combination of protogon, and its products of decomposition (stearic acid, phosphoglyceric acid, and neurin). Neubauer obtained it from oleic acid by simply placing small portions of the acid side by side on the slide, and covering them with a drop of liquid ammonia. He likewise obtained it from a mixture of caproic and capric acids and ammonia. Hence we may conclude that myelin is a special form derived from modifications of fatty substances." †

Having repeated this experiment of Neubauer with oleic acid and ammonia, I can testify to the production of the various forms of myelin, paler in colour, and with a prevalence of fringed forms. When gentian violet is allowed to run into the preparation, some of the particles are stained to a deep blue, as occurs with sputa; but the greater number are stained violet or brownish red, these particles having a tendency to become more liquid and run to the edge of the portion under examination. The solution of iodine in potassic iodide, allowed in like manner to flow into a preparation, dissolves all masses of myelin, while it permanently stains those of the sputa. But if, instead of allowing the ammonia to run by capillary attraction between the slide and the thin cover, a drop of it is allowed to fall on the oleic acid without the cover-glass, the whole becomes a saponaceous mass, wherein no trace of myelin can be seen, it being dense and too opaque.

* Henle, *Tratt. di Anat. gen. edit. Levi*, Venice, 1845, Vol. II., pp. 144—45, Pl. IV., Fig. 5, in which are drawn many of the forms taken by myelin when escaping from incisions in the nerve-fibres; magnified 300 diam.

† F. Selmi, *Enciclopedia di Chimica*, Turin, 1873, Vol. VII., pp. 883—84.

## Myelin in Whooping-Cough Sputa.

It has been seen that myelin is not always distributed uniformly nor to the same extent in certain sputa. If on emptying the sputa from the sputa-cup, striæ of a hyaline mucus are noticed adherent to the bottom or to the walls of the vessel, it probably will be found that myelin is more abundant in them, as myelin will render such portions, where it is in larger quantities, considerably more viscid than the rest. But if a fresh portion of the sputa is examined in its natural state, after a very short time the particles, filaments, and free granules of myelin, and, above all, the masses, will rise to the surface of the specimen, flowing at times over the lower matter to the edge of the thin cover-glass, following the currents produced in the preparation caused either by greater evaporation going on one side than the other, or by air-bubbles, or by motion imparted to the mass under the hands of the manipulator ; so it is clear that myelin is much lighter than the rest of the matter of the sputa, and being once free rises to the surface. In a conical glass or in a test-tube myelin will very soon rise to the surface, as the fatty globules do in milk, of course with the exception of such particles that are in close adhesion to the epithelium cells or firmly embedded in the mass of the mucus. When to a dense mass of mucus some diluent—as, for example, a solution of chloride of sodium—is added, very soon we have a central mass of viscid matter surrounded by the more fluid diluent, into which the lighter particles and granules of myelin will flow, in the same manner as all the lighter and more fluid particles make their way to the edges of preparations.

Such particles and granules that remain behind are those that are deeply embedded in the mucus. They may be seen principally in the striæ, moving at times along the course of these till stopped by the crossing and closing up of the tiny channels formed in the viscid mass. These particles would easily escape observation in the natural state, but when stained become distinctly visible, especially if gentian violet is used, for the edges of the striæ take a more or less blue tinge, and the imprisoned myelin can hardly be overlooked. The granules of myelin adhering to the ellipsoidal cells of epithelium are well shown, too, by staining the mass with gentian violet on account of the colour imparted to such cells. In fresh preparation, especially in the unstained sputum, after some little time, these granules of myelin will leave the cells of epithelium and float off into the surrounding fluid.

The general appearance of the masses and particles of myelin, in its free state or in sputa, is well described in the passages given from Beale and Ranvier; but it would be impossible to give a complete idea of all the many forms in which it may be found.

In the natural state the masses and particles have the colour of olive oil at the edge, but the central portion seems to be of a light greyish tint, but this changes under the microscope, as the focus is altered by the slow motion. These are shown in Fig. 1, *a*, *a*, *a*, *a*, and *c*. When the mass is in repose, or is moved without distortion, it is usually marked by concentric striæ. But if, as in *c*, an extremity, *d*, of a mass of myelin is caught by some foreign body, a current of the containing fluid will push forward the rest as a rounded head, as in *e*, leaving a narrow neck linking it to the part held firm by the obstruction. The neck becomes thinner and thinner as the progress of the moving portion of the mass continues; the striæ in this portion being distorted more and more, taking at last the appearance of closely packed threads. The neck may be stretched till its length becomes some ten times that shown in the figure, and is proportionately thinner, and at last snaps in two. These broken bands, when stained by gentian violet, are quite distinct; at times they seem to become solid, as in *b*, or form new smaller globules, as in *b* above, on the left. Near the middle of the group shown may be seen a small portion of myelin with two wings, and which in the preparation seemed endowed with a rapid vibratory motion. These threads and filaments are, of course, hardly visible when left unstained.

Besides the so-called masses, many of which are much larger than that shown in *c* (mag. 400 diam.), there are found aggregations of particles, as in *a* on the right, of the most varied shapes and sizes. Such united particles rarely coalesce, but take by their mutual pressure a thousand different shapes.

In the unstained state the granules of myelin are even less visible than the filaments; in fact, are too minute to be visible. A solution of iodine, picric acid, and fuchsine are a great help, as we shall see in the sixth section; but gentian violet is of a far greater value, as it gives to the granules and particles of myelin a more or less blue tinge, whilst bacteria and other morphological particles become decidedly violet under its action. This is shown in Fig. 1, where *b*, *b*, *b*, *b*, *b* should be compared with *f* and *h* in the centre and right of the same figure, and then with *f* and with the stained bacteria in Fig. 2, especially with *s*, where the isolated bacteria and the beaded forms are tinted with violet, while the granules of myelin which interpose are stained pale blue. Without this process of staining, the granules of myelin would easily be passed over or mistaken for minute micrococci.

### Myelin in other Sputa.

It must not be thought that myelin, as described above, is exclusively found in the sputa of Whooping-cough, for it is well known that traces of it are found in all kinds of sputa.

(*a*) I have frequently examined the sputa of persons in the best of health, both during the day and immediately after rising in the morning. In such sputa the ellipsoidal cells of epithelium are constantly found covered with myelin, as well as some few particles or free granules of myelin. Nevertheless, I have never found such profusion of masses as in typical cases of Whooping-cough. In all probability, the free granules of myelin in healthy sputa may be held to be simply proportions that have become detached from the epithelium cells, whereas in cases of Whooping-cough the masses are out of all proportion with the number of cells. Again, the proportion of such sputa in healthy subjects is very small. During the whole twenty-four hours the entire quantity would hardly be altogether more than one single expectoration of sputa in Whooping-cough, so that the total amount of myelin expectorated by a person in health is but a very small fraction of that produced in Whooping-cough.

(*b*) In acute attacks of bronchitis the ellipsoidal epithelium cells are found covered with myelin and also granular masses of the same, as Bizzozero's * note testifies, but this only in very small quantities as compared with that met with in cases of Whooping-cough. And then it must be remembered that even in cases of a severe character, when the ellipsoidal cells of epithelium are want-

* Bizzozero, *Manuale di Microscopia Clinica*, Milan, 1882, p. 154.

ing, no myelin whatever is seen. So also in chronic bronchitis myelin diminishes and disappears altogether, as the ellipsoidal epithelium cells diminish or fail to appear.

(*c*) In cases of inflammation of the lungs, in which sputa are so frequently examined, it is well known how small the amount of myelin is, though in such cases the above cells are more than ever abundant.

(*d*) In no genuine case of pleurisy has myelin ever been found. The mass of the sputa may be said to entirely consist of mucus overrun with bacteria, which are, for the greater part, to all appearance, capsular.

(*e*) In phthisis, where the sputum is most often examined, myelin is rarely met with, and when found is only present in minute granules, and can only be discovered by the use of gentian violet in fresh expectorations. Only one exception to my experience do I know of—that mentioned by Dr. Beale *, in which case the sputa must have contained, according to his report, an amount of myelin approaching that which I have found in Whooping-cough.

I am therefore inclined to think that, both in health and disease, there may be a continual, though very limited, flow of myelin in the air-passages, as is found in the liver and other organs according to Beale. It is a very different matter when we consider the sputa in cases of Whooping-cough.

In these cases we have no transitory formation (as in the above case spoken of by Beale), but the production of several grammes of myelin within the twenty-four hours, and, what is much more, a daily repetition of it for some months. If we suppose some twelve paroxysms a day, and the amount of sputa at each paroxysm to be about five grammes, there would thus be sixty grammes of sputa expectorated; and the preparations described above show that of this amount a very large proportion consists of myelin. What is the cause of this appearance?

I will here briefly say what at once came to my mind in answer to this question. First, we have a disease in which an extraordinary formation of myelin is produced; and secondly, a tissue affected which is most rich in myelin, namely, the nervous tissue.

* Beale, *loc. cit.*, p. 282, and Pl. XL., Fig. 8.

The very character of the disease (Whooping-cough) is the intense excitement of this tissue. May it not be supposed that this rapid and intense deterioration or disintegration of this tissue gives the key to the solution of the question?

Though Beale does not consider the existence of an alveolar epithelium sufficiently proved, nevertheless, he admits that particles of myelin are found in the air-passages and air-cells of the lungs. Such are shown in *i* in Fig. 1 * of the plate, so that this abundance of myelin might well be thought to be merely the result of physiological action, the increased excitement of the nerves of the part affected bringing about a disintegration of the medullary sheath.

THE IMPORTANCE OF THE MYELIN IN WHOOPING-COUGH.

(*a*) There is no doubt that the far greater part of medical authorities hold Whooping-cough to be due to either a direct or reflex nervous action.

Cullen, Brouzet, Pinel, and numerous others are of opinion that Whooping-cough is caused by an irritation of the mucous membrane of the gastro-intestinal tract, and Padalme adds to these causes irritation of the lungs and diaphragm. Watt, Alcock, and Laennec consider the disease to be a *spasmodic variety of bronchitis ;* and Brousseais thinks, in addition, that an increased irritability of the mucous lining of the inflamed bronchial membrane is another cause. Trousseau and Richard de Nancy believe it to be but *bronchitis grafted on a neurosis.*†

Giuseppe Frank quotes the cases of autopsy made by Hermann Kilian, who in the bodies of fifteen boys, victims of Whooping-cough, found the pneumogastric nerves highly inflamed.‡

G. Pietro Frank places Whooping-cough among the nervous diseases.§

Copland, in his *Dictionary of Practical Medicine*, states that he has found in nearly all the bodies of those dead from Whooping-

* Dr. Beale, *loc. cit.*, p. 389, Pl. XXI., Fig. 6.

† Barlow, *Lancet*, 1886, Vol. I., p. 870.

‡ G. Frank, *Prax. Med. Univ. Præcept.*, Lipsiæ, 1823, Part II., Vol. II., p. 833.

§ G. P. Frank, *Epitome di Medicina Pratica*, Chiaverini edition, Naples, 1833, Vol. XIII., pp. 153, etc.

cough severe inflammation of the cerebral membranes and of the medulla oblongata.*

Hagenbach, in above-mentioned monograph, mentions many authors of the same opinion. "The seat from which spring the attacks of Whooping-cough (says Biermer) has been placed in the vagus and recurrent nerves, as well as in the phrenic or in the intercostal nerves, in the sympathetic and in the solar plexuses. Webster (*London Med. and Phys. Journal*, December, 1822, p. 478) holds "that the actual seat of the complaint may be in the head, and that the affection of the respiratory organs is only to be considered as a secondary effect." Hufeland and others attribute it to excitement of the respiratory nerves. Friedleben (*Archiv. für Physiologische, Heilk.*, Volume XII., 1853) thinks it due to a compression of the vagus and recurrent nerves by the swelling of the glands of the bronchiæ and tracheæ. Bouchut thought it to be a complication of catarrh and nervous affections. Henoch (*Beiträge zur Kinderheilk. Neue Folge*, 1868) thinks it to be an affection stimulating in some unexplained manner the nerve-centres of the medulla oblongata, producing spasmodic coughing. It is true that Hagenbach, relying on the observations of Letzerich, thought Whooping-cough a mycotic catarrh, but admits the presence of a nerve stimulus given to peripheral ends of the superior nerve of the larynx. †

Rilliet and Barthez, in their treatise of *Pediatrics*, have placed Whooping-cough as a class of disease between the acute exanthemata and the neurotic diseases. The following observation of these authors are worthy of great consideration :—" Whooping-cough may show itself at any period of infancy. We have observed it in a new-born child whose mother had suffered from Whooping-cough a month before her confinement. The *quintes* showed itself in a most violent and distressing manner on the day of birth." ‡

* J. Copland, *Dictionary of Practical Medicine*, Part V., Art. Whooping-cough, London, 1838.

† Gerhardt, *loc. cit.*, pp. 479—80.

‡ Rilliet and Barthez, *Traité Clinique et Pratique des Malad. des Enfants*, Paris, 1853, Vol. II., p. 644.

Bouchut has known cases where Whooping-cough developed itself in a child but two days old, but he says nothing of the *quintes.** This sudden appearance of the disease distinguishes it at once from such maladies as are contracted from living germs which require for development a period of incubation. We cannot suppose any infection communicated during interuterine life, for such infection would seem impossible in a disease so local as Whooping-cough, and is only known in infections more general in their nature, as in syphilis, acute exanthemata, etc.; whereas any disturbance of nerve excitability may well be transmitted by the law of sympathy from the nerves of the mother to the homologous nerves of the fœtus. Henning was of opinion that even the sound of Whooping-cough may cause in another subject a stimulus of the bronchial nerves.† Niemeyer, though he held Whooping to be an infectious cough, speaks even *of using the whip* in this malady.‡

Delaberge, Monneret, and Fleury, in their compendium of *Practical Medicine*, mentions a case, referred to by Gendrin, in which a persistent cough, with all the characteristics of Whooping-cough, was observed by Dupuytren and Husson to result from an operation for opening the parotid gland.§

" Unless we reject the evidence of well-established cases, it is impossible," these authors add, " to refuse to look upon Whooping-cough as a nervous affection, more especially if we consider—1st, That the respiratory parts present no alteration, or if any are present they are of so varying a character as not to be considered a cause of the disease; 2nd, That the course of the symptoms is evidently remittent, but with such an absence of fever, when any complication does not exist which is not observed in inflammations of a general or specific character; 3rd, The sudden cessation and return of the spasms of coughing, brought about by emotion or by change of place, are phenomena that point to nerve excitement,

* Gerhardt, *loc. cit.*, pp. 475—76.

† *Ibidem*, p. 480.

‡ Niemeyer, *Patologia e Ter. spec. int. Versione Cantani*, Milano, 1863, Vol. I., p. 38 (in a note).

§ Delaberge, Monneret, and Fleury, *Compendio di Med. Pratica, Versione it. Firenze*, 1851, Vol. VI., p. 314.

and not to inflammations that require time to run out their course; 4th (and lastly), That rapid regaining of perfect health, and the healthy condition of all functions in mild cases, the resistance of the malady to treatment, and the inefficacy of the usual remedies prescribed for inflammatory diseases, the success of narcotics and antispasmodics, are all circumstances met with in nerve affections, but not in diseases accompanied by inflammation." *

Grisolle repeats the same arguments for including the Whooping-cough in the class of neurotic diseases. †

Jaccoud also admits the specific excitement of the nerves in Whooping-cough. It is proved beyond doubt by Rosenthal's researches that a centripetal stimulus given to the superior laryngeal nerve determines a relaxation of the diaphragm, the closing of the glottis, and a convulsive expiration. That is, the phenomena that distinguishes a convulsive cough from others altogether catarrhal, are explained by an *irritation of the superior branch of the vagus nerve, produced by a reflex action on the medulla oblongata, thus arresting inspiration and causing a spasmodic expiratory action of the glottis.* ‡

West believes Whooping-cough to be *a bronchial and nervous affection.* He mentions the fact that the pneumogastric nerves have been found on examination to be reddened, swollen, and softened, but only in exceptional cases. According to him, Albers of Bonn in forty-seven autopsies found in three cases only the vagus reddened on the right side, and in one case on the left, which might have been merely the result of position. Only in one case out of twenty-four, he found the nerve on both sides of a higher colour than in the normal state. Hence, he concluded that such changes were simply accidental or due to alteration after death. He does not make any allusion to the superior laryngeal nerve. §

Certainly, the sudden beginning and ending of the spasms, which he describes, plainly point to a nerve affection rather than to infection by living germs. || But not to prolong this matter, I refer the reader to the respective authors.

* *Ibidem*, p. 336.

† Grisolle, *Tratt. elem. e prat. di Pat. int. vers. Del Corso*, Livorno, 1853, p. 877.

‡ Jaccoud, *Tratt. di Pat. int. vers. Borrelli*, Napoli, 1872, Vol. I., p. 751.

§ West, *Lezioni sulle malattie dell' infanzia e fanciullezza, vers.* Blasi, Milano, 1869, pp. 498—99.

|| *Ibidem*, pp. 476 and 493.

In the first paragraph I mentioned Michael's and Sonnenberger's opinions as well as that of others, who hold that Whooping-cough is but a reflex nerve affection.

In a treatise on the pathology and treatment of Whooping-cough during last May, Genser, without entering into any new or microscopic researches, combats the practice of nasal insufflation recommended by Michael, and advocating in their place the use of antipyrin, not as a parasiticide, but on account of its sedative action on the nerves, reducing or suppressing reflex action in them. This shows how much he considers nerve affection has part in the disease.*

Cohnheim and Habershon likewise consider Whooping-cough to be of the nature of a nerve derangement. Habershon, moreover, thinks it proceeds from some derangement in the function of the pneumogastric nerve.†

The hypothesis of the breaking up of the medullary sheath, replete with myelin, of the nerve-fibres in the air-passages, includes the supposition of some morbid excitement of these nerves. Nerve-fibres, with the exception of the *fibres of Remak*, are invested with a medullary sheath, forming a layer of considerable relative thickness between *Schwann's sheath* and the *axis-cylinder*, being interrupted by the constrictions of Schwann's sheath at regular intervals; the fibres of Remak are readily distinguished among the medullated nerves by the absence of the medullary sheath and by their anastomosing plexuses.‡

Here we are confronted with a difficulty of explaining the passage of the myelin, escaping through such alterations from the nerve-fibres into the interior of the air-passages. It would appear that myelin must be included amongst the colloid rather than the crystalloid substances; its power of passing through animal membranes would then be very little.

But such a difficulty would militate chiefly against that portion

* Genser, *Zeitschr. f. Ther.*, No. 9, 1888; *Riforma Medica*, July, 1888; and *Morgagni*, Part II., No. 39.

† Cohnheim, *Lez. di pat. gen.*, Vol. II., Naples, 1882, pp. 149, 150. Habershon, *Lez. sulla pat. del n. pneumogastrico*, Milan, 1879, pp. 19, 20, and p. 30.

‡ Ranvier, *loc. cit.*, p. 772, etc.

of the myelin which is supposed to be formed at the expense of the nerve-fibres which surround the alveoli, or the terminations of the ultimate bronchials; where the existence of a mucous membrane, properly so called, cannot be demonstrated on the internal surface of the membrana anista. It is not the same with the whole of the remaining bronchial tree, the trachea and the larynx, which are invested with a mucous membrane very rich in nerve-fibres.

In the epiglottis, according to an example drawn by Beale, the network of nerves lies on the surface of the mucous membrane immediately underneath the epithelium.*

In the mucous membrane of the larynx and trachea it is less superficial, but in the bronchi it again comes to the surface with a thinning of the mucous membrane.†

Hitherto we have only expressed a doubt as to the origin of the myelin in the sputum of Whooping-cough, and we refrain from entering into further disquisition on the subject, for which there would be no occasion, except the fact stated by us of the extraordinary abundance of myelin in such sputum, was confirmed by further and more authoritative observations.

(*b*) The abundance of the myelin once proved will also give us the explanation of the singular character which the sputum of Whooping-cough always presents. Its viscosity, which presents an impediment to expulsion, does not depend on the condensation of the mucus, nor has it anything in common with the thick and tenacious mucus which is commonly met with. The sputum of Whooping-cough is, on the contrary, fluid, thin, and transparent.

* Beale, *loc. cit.*, p. 384, Pl. LX., Fig. 2. The same in the Lectures on the Structure of Simple Tissues, etc., Borrelli, ed., Naples, 1865, p. 284, Pl. XV., Fig. 95.

† Kölliker, *Elém. d'histolog. hum.*, Paris, 1871, pp. 510—529. Sappey, *Traité d'Anat. Descrip.*, Paris, 1889, tom. IV., p. 439. Stohr, *Ist. d'istolog.*, etc., Naples, 1887, p. 164. Concerning the degeneration and reproduction of nerve-fibres, the travelling of the myelin particles, and the very slow reproduction of the medullary sheath, see, amongst other authors, Tizzoni, *Sulla patolog. del tessuto nervoso*, etc., *Arch. p. le Sc. Mediche*, Turin, 1879, Vol. III., No. 1; Alonzo, *Sulla degeneraz. delle f. nervose*, etc., *Arch. citato*, Vol. XIII., 1889, p. 229, etc.

Now, these properties, combined with its viscosity, are perfectly understood if they relate to the myelin.

Further, the presence of a notable quantity of myelin will furnish a critical diagnosis in certain cases.

Rilliet and Barthez have established the great difficulty of a differential diagnosis in grave cases of pulmonary complications which are evolved in the early stages of the disease, and are protracted in the later stages, resembling *capillary bronchitis with fits of coughing (with quintes).* The differential diagnosis between Whooping-cough and tuberculosis of the bronchial ganglia is yet more difficult.*

Hagenbach, in order to make the diagnosis more certain in doubtful cases, considers it indispensable to be present during a paroxysm, and if required to provoke it with some stimulus, Whooping-cough being liable to be confounded with capillary bronchitis, pulmonary phthisis, tuberculosis of the bronchial glands, hysterical cough, and relaxed uvula.†

Now, in all these cases or others similar, the microscopical examination of the sputum, whenever there is a great abundance of myelin, will easily solve the question.

## Researches on Expired Air.

We shall see in the sixth section that the minutest particles of myelin—so minute that they can scarcely be observed unless stained—are animated by a lively molecular motion. Its extreme tenuity and motobility in liquid currents has already been noticed. It cannot be thought impossible, nor even improbable, that these minute particles of myelin may be expelled with the expired air, especially during the attacks of coughing, and be diffused in and remain suspended in the surrounding atmosphere, for such particles are less, both in weight and volume, than the small bacteria found in the air.

For want of better apparatus, I have made use of large plates of water, to which has been added a small quantity of phenol, and

* Rilliet and Barthez, *loc. cit.*, p. 639.

† Gerhardt, *loc. cit.*, pp. 495—496.

kept them some ten days in the rooms of patients. But on examination of the flocculent deposit in this water, which was poured into a conical glass, I have not found either particles or granules of myelin. Many living amœba were found; also spores (of which the greater part was very similar to the spores of the larger fungi observed in the sputa), as well as two forms of bacteria. One of these was dumb-bell shaped, and similar to the types *d* and *e* in the plate, Fig. 2, and most likely the same as that found by Poulet; the other showing spotted granules very much after the manner of the supposed bacillus of typhus fever. I need not give a full list of other substances observed, such as pollen and starch-grains, etc. In the doubt that the particles of myelin might have remained floating on the surface of the water, this, too, was examined without any discovery of myelin.

But this examination was too imperfect to enable me to draw any definite conclusion. I leave others, better equipped with suitable apparatus, to settle this point, and to enter into the question whether such a diffusion of myelin in expired air, if proved, might not be a means of spreading the disease.

To ascertain, moreover, whether vapours could diffuse the granules of myelin, some sputa were diluted with distilled water and boiled in a test-tube for some two or three minutes. A glass slip was applied to the mouth of the tube and held there by a spring. The examination of the dewy film in the glass slip did not reveal any trace of myelin, but I observed some minute globules, oleaginous to all appearance, which seemed to tend to unite in larger globules, and were not at all stained with gentian violet. I cannot say that these were formed in any way by the boiling of the myelin, but of this I am sure, that the quantity of the myelin in the boiled liquid was diminished.

It will not be out of place here to mention an incident which took place during one of these examinations. Having inadvertently taken some ordinary cistern-water in place of distilled water, I found in one of the preparations a fungus as shown in its natural state in Fig. 3*a*, magnified 400 diameters. I have had occasion to speak of this fungus in another communication to the Society in reference to certain diplococci, similar to the gonococci of Neisser, and observed by me in a case of cancer of the bladder.* The spores of this fungus, being light, float on the surface of liquids and are taken up by pipettes, or glass rods, and deposited on test-tubes or on glass slips for examination. Very likely the warmth from the boiling favoured the germination of the fungus, as shown in the figure, for in all the many preparations examined I have only found this fungus in germination once before, in a dried portion of sputa of a consumptive patient.

The casual germination of the fungus in this sputum, diluted and boiled as it was, offers by analogy an explanation of the germination of the fungi, *k* and *n* in Fig. 3, the sole difference being that the fungus *a* was deposited in the sputum after expectoration, and those in *k* and *n* received internally.

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§ 4.

## OBSERVATIONS & CONSIDERATIONS ON THE BACTERIA.

IN the second section I have briefly described the principal forms of bacteria and bacilli observed in the sputa in Whooping-cough. It will now be well to take a more systematic view of their forms, and compare them with others found in different sputa and other substances, not with the object of raising the question of their pathogenic power in engendering disease (which I do not uphold), but to show the value of their clinical importance in the diagnosis of the disease.

### Bacteria and Bacilli found in Whooping-Cough.

All the forms of bacteria represented at *a*, *b*, *c*, *c'*, *d*, Fig. 2, with exception of *c'*, are very frequently met with, and can hardly be said to differ much from *Bacterium termo*. They are found in all kinds of sputa, and in substances of widely different character; and it should be noticed that they do not differ from the forms *e* and *f*, which are found covering and infesting the buccal epithelium cells and the salivary corpuscles, but do not in any way attack the vibratile epithelium cells, nor the pavement cells of the air passages.

* *Sopra un diplococco analogo al gonococco del Neisser, rinvenuto nella orina in un caso di carcinoma della vescica.* See Tom. XLIII., *Atti R. Accad. Med. Chir.*, 1889. The discovery of such curved forms in diplococci, even in the sputa of Whooping-cough, is fatal to the supposition of their being a specific characteristic of any disease.

This was observed before by Barlow, as stated in the first section of the memoir,* who was of the opinion that the epithelium cells infested by these bacteria were only those of the larynx and pharynx; but comparing my observations on the sputa of Whooping-cough with those on other sputa, healthy or otherwise, I cannot in the least agree with the illustrious doctor of Manchester. It is quite sufficient to compare these pavement cells, large and thick, with those of the saliva, to assure oneself that they proceed from the buccal cavity, and not from either the pharynx or larynx. Moreover, we must remember that if the epithelium cells of the larynx or pharynx become detached by a supposed specific pathogenic action of these bacteria, they could not in any possible way undergo any regeneration to allow them fully to develop, especially considering that the mucous membrane would be in a high state of irritation. How then can we account for the constant presence in the sputa of such fully developed epithelium cells, and which are always infested by swarms of these bacteria?

This fact is to me a most convincing proof of the buccal origin of these bacteria. For if they originated in the sputa, they would doubtlessly be found more frequently among the epithelium cells of the air passages than among those of the mouth, or in the salivary corpuscles, or at the very least as often among the first as among the latter. The former could hardly be attacked by the bacteria in the short period elapsing between the expectoration and the immediate examination, and such immediate examination of the sputa shows that while epithelium cells of the mouth swarm with such bacteria, the epithelium cells of the air passages are entirely free from them.

We must conclude, therefore, that the germs of such bacteria found free in the sputa come from the mouth, having been inspired with the air, and lodge within the entrance of the air passages, after the manner in which Dante describes the fate of the souls of suicides:—

"Into the wood it falls, at no fixed spot;
But where blind fortune haply shoots its bolt,
There like a grain of corn it germinates."

—Inferno, XIII., *D. Johnson's trans.*, 1867.

* See page 2.

This simile, though it may appear to be out of place in a scientific treatise, is an apt one, not only in regard to the bacteria *a*, *b*, *c*, *d*, but to all other bacteria or bacilli found in the sputa; for there are none, we may safely say, that are not often found at the same time in the epithelium cells of the mouth, or in the saliva, and the germs of which may not have been introduced into the air passages by the currents of the air as it is inhaled.

This view is not only in complete accordance with the observations of Barlow, but with those also of Jansen and Tschamer, and with the deductions which Cornil and Babes drew from the observations of Bürger, as was noted in the first section.

As before stated, it is not my intention to enter into the pathology of the subject, but I should like to point to one fact, which I believe has not been referred to before by any one, and it is this :—(it seems to me) that there is evidently a great disproportion in the power of producing disease attributed by many to bacteria on the one part, and their extreme smallness on the other.

The bacteria *a*, *b*, *c*, and *d* are of medium size as compared with other bacteria; now if we take a single grain of wheat, weighing some five centi-grammes, we shall see that it is about half as long again as one of these bacteria, on the scale of the figure; twice as broad and twice as thick, and would be in volume equal to that of six bacteria, and in weight some eight times that of a single bacterium. A grain of wheat if put into water sinks at once to the bottom, whilst the bacterium will float on the surface. Now the bacteria in the figure are magnified to 2,500 diameters, or 6,250,000 times superficially, or 15,625,000,000 in volume. So we must conclude that this number of bacteria would equal some eighth part of the five centigrammes, or the weight of one grain of wheat. In other words, to make up five centigrammes of bacteria, we must have 15,625,000,000 bacteria multiplied by eight; that is to say, 125,000,000,000. Hence in one centigramme we may say there must be some one hundred and twenty-five billions of bacteria. These calculations will not appear to be exaggerated when compared with those of Naegeli, which, as is well known, are some twelve times greater.

How far such extreme smallness can be reconciled with the power of producing disease attributed to these bacteria must be left to pathologists to decide. We hasten to reconsider the clinical and semiological questions.

Enough was said about the bacteria *c* and *d* in the clinical notes of the first case. In *c*, above, and in *d* are seen the dumb-bell like bacteria in series; while in *c*, below, are seen the bacteria, cylindrical in shape, somewhat swollen in the middle, and arranged in shorter and more sinuous threads. Now, it seems to me that from this last form may be derived the other forms, *c' c'*, which are magnified 860 diameters.

I have occasionally come across this chain-like series, shown in *c' c'*, in the sputa of consumptive patients; it is stained with gentian violet. The individual articulations or bacteria differ but little from those shown in *c*, below, but they are somewhat larger (in the figure they are shown too long), inclosed in a sheath with a well defined outline, and at the points of contact they seem to coalesce, for no septa, but only restrictions—such as are seen also in Fig. *f'*—are discernible.

Of the bacteria *e*, *f*, *l*, the forms *e* and *f* are nearly identical with those shown in *b* and *d*; but are met with, sometimes in large numbers, enclosed in a sheath, whether they are within or without the epithelium cells. They thus have a distinctive feature in this sheath, real or apparent, in common with the bacteria of the types shown in *f'*, *l*, *l'*, *o*, and *p'*. The sheath seems to be better defined when the specimen is stained with methyl violet, and still better when the medium is less refractive. I have been able, even in water, to detect this semblance to a containing sheath; but glycerine, when it once permeates the preparation, renders the sheath at first almost invisible, and after some hours totally so. But in air it is quite the reverse. In those parts which, by evaporation of the medium, are left dry on the slide, the sheath becomes at first quite distinct, though eventually, when quite dry, both the sheath and the bacteria become a confused mass.

Many bacteria, morphologically identical and with the same apparent sheath, are found in the saliva and in the accompanying buccal epithelium cells. I have met with them in all kinds of sputa, and believe them modified forms of the same kind. So in *l* and *l'*, on the right, there is seemingly the beginning of a union of the extremities, and a tendency to take the form of *e* and *f*.*

* See Fig. 33, p. 76, in the previously cited manual by Jaksch.

I shall have to refer to other bacteria with a similar apparent sheath.

The bacteria, *g*, *p*, and *p'*, curved and coupled together, closely resemble the so-called *Gonococci* of Neisser, supposed to be the specific characteristic of virulent Blennorhœa, and are found, though in small numbers, in saliva and occasionally in the sputa of Whooping-cough. In Fig. *l* we have a similar but larger form, intermediate between the curved forms seen in *g* and *p*. I fail to see why these forms, though varying in size, should be held to be distinct species.

I must draw attention to the curved forms shown in *p*. They are linked in a chain-like thread, together with bacteria of about the same size, but straight, and, in all but their size, identical with those shown in *b*, *d*, and *f*. Now, it seems quite inconceivable that such a chain or beaded thread of bacteria could be made up of individuals of totally different species ; in other words, that a complex organism—a truly Horatian monster, as we should have in the forms *p*, *p*, could possibly be the outcome of the fortuitous grouping of organisms of quite different species. We must, I think, come to the conclusion that the curved and the straight forms in *p* belong to the same species. That the straight forms are identical with those in *b*, *d*, and *f*, is established by the fact that they both are found living on the buccal epithelium cells and have the same dumb-bell shape, and take the same sheathed forms, which the curved forms do not. This shows, on one side, that the semblance of a sheath does not of itself constitute a specific difference ; and on the other, that all the so-far mentioned forms, from *a* to *p*, in all probability, are but modifications or stages of development of one and the same species.

Fig. *p'* shows two bacteria crosswise enclosed in a common transparent capsule ; but they are found oftentimes singly, or three or four together so enclosed. The single or isolated forms cannot be distinguished from those shown in *l'*, above, where these are seen more or less foreshortened, for then the transparent or semi-transparent division is invisible until the bacteria is changed in position. Again, the bacteria shown in *p'* may take the appearance of diplococci, after the style of *f'* and *l'*, with disjointed members, whenever they are seen, not sideways, as in the figure, but end-ways, for their club-headed extremities have the same apparent shape as the rounded diplococci.

The bacteria of the type shown in *p* are sometimes met with in quite large agglomerations, mingled with other very minute bacteria of the same shape. This we saw in the second section. These are very numerous in sputa in phthisis, inflammation of the lungs, and in other sputa. With methyl violet they take sometimes a dense blue, sometimes a violet tint, both tints showing at times in the same preparation. I shall have occasion later to speak of some probable cause of the appearance of the sheathed or capsular forms.

The form presented in *h* was, in linear dimensions, almost double the size of the so-called pneumococci, as will be seen if my drawing be compared with Fig. 13 of Pl. XV. in the quoted work of Cornil and Babes, under *a*, *b*, and *k*; bearing in mind that the drawings of these authors are on a scale of something less, viz.: 2,000 diameters. In my drawing a distinct outline seems to show the extent of the capsule or sheath.

I was sorely perplexed as to the nature of this body till, in a case of pleurisy, I met with a large number of similar bodies of about the same size as those I had found in Whooping-cough, with this difference, that those in the case of pleurisy were of various shapes, some being elongated and constricted in the middle, others ellipsoidal, rounded, or altogether irregular in outline. I was soon convinced that such bodies were but small pus or mucus corpuscles, having a nucleus highly stained, the rest pale blue by osmosis. I had used as a dye a fresh preparation of methyl violet, and concluded that the body *h*, stained with gentian violet, was of the same nature, and had taken by mere accident the appearance of a diplococcus with a sheath, compressed in one direction and lengthened out in another by the threads of the mucus in which it was held.

The beaded forms of bacteria *i* and *k* have nothing to distinguish them from those found in urine, blood, and many other substances undergoing decomposition. In the form *i* germination seems to be carried on at one end only, and the larger members, which may be taken to be older bacteria, are more deeply stained. In the form *k* increase seems to proceed at either end, but no specific difference can be seen between the two types. It is easily conjectured that these beaded forms may develop into the chain-like threads in *c* and *d*.

From my observations of these bacteria, or rather bacilli, of the beaded forms, and the difference of colour imparted in staining to the young and to the older members, I am led to think that the germinal matter predominates in the younger ones, and this is less readily acted upon by aniline dyes; whereas the older members become invested with some covering which takes up the stain more easily, as we often see is the case in frustules and filaments of vegetable origin. For with a parity of development I find the contrary takes place: the smaller bacteria are more readily stained than the larger forms.

For the sake of brevity and clearness I will defer till later the description of the Figs. *m* and *n*.

In Fig. *o* we have one of the curved forms of bacteria enclosing a pale-blue substance. It greatly resembles the curved forms *g* and *l*, and is not very unlike those contained within the bacillus *n* on the lower left side of the figure. I have not met with many specimens of this type, and of the type shown in *o* above still fewer have come under my notice. This last is a true diplococcus enclosed in a capsule, and its nature is unknown to me, for the internal matter as well as the rest is stained.

The bacteria *q* is rarely seen in sputa of Whooping-cough, but is more frequently found in other sputa. It has no specific importance, being, as we shall see, but an undeveloped *Leptothrix*.

The form *r* is of very rare occurrence, but from both these we can see that the longer forms of the bacteria, single or linked, as in *q* and *r*, do not differ substantially from the more rounded forms, as shown in *l* and *y*.

At first sight, the beaded form *s* seemed to me to bear great resemblance to the bacillus of Koch, *Bacillus tuberculosis*. They are found in many various kinds of sputa. These beaded forms may be called in truth bacilli, for when magnified to some 400 or 500 diameters, they appear to be one entire, unbroken thread, as also, under the same power does the bacillus of Koch. Under higher powers, both one and the other show well their moniliform structure. This is well seen if my drawing in the Plate (2,500 diam.)

is compared with Figs. 12, 13, and 14 of Plate XXII. of Cornil and Babes, bearing in mind that these last are only drawn to 1000 diameters. Such beaded forms did not stand the test with dilute nitric acid (one part acid to three of water), and alcohol as we saw in the second section. I shall have to refer to this again in another place.

These beaded forms and the bacilli of the same type, are frequently observed in saliva, and seem to predominate both in saliva and sputa around or within the buccal epithelium cells, as I observed in the seventh case of Whooping-cough. I have good reason to think that these beaded forms are substantially the same as the chain-like threads in *u*, *u*.

It will appear from this short description, firstly, that all bacteria found in Whooping-cough sputa can be reduced to a very few species, identical with those found normally in the mouth and perhaps also in the nose ; secondly, that until experiments in culture and inoculation, *invariably and for a long period of years*, give us exact and conclusive results to the contrary, we are right in maintaining that none of these forms are specific causes of disease, and that Whooping-cough is not rendered contagious by bacteria.*

## Bacteria and Bacilli found in Healthy Sputa.

In healthy sputa, collected in the manner indicated in the foregoing section, the following bacteria are found:—the dumb-bell forms, *b*, *d*, *e*, and *f*, the beaded forms, *i* and *k*, and those shown in *g* and *l*. I have observed, too, in the sputa of healthy persons, colonies and numerous samples of the straight forms of bacteria, *p*, *p*. Even in the sputa examined at once, these bacteria were found in greatest numbers in such portions where there was

* This second conclusion is entirely supported by Mackenzie, of Edinburgh, in his monograph on sputa in general, who, by the way, makes no mention of myelin.—G. Hunter Mackenzie, *Le crachat dans ses rapports avec le diagnost.* ; etc., Paris, 1888, page 21. Such conclusion is thus spoken of by Sternberg:—"Dr. Vicentini very properly arrives at the conclusion that no one of the bacteria observed by himself, or by those who have preceded him in examining the sputa in cases of Whooping-cough, has been demonstrated to bear an etiological relation to this disease."—*Proceedings of the American Microscopical Society*, July, 1893, p. 158. [F. V.]

a mixture of the saliva; these portions were marked by the abundance of buccal epithelium cells, salivary corpuscles, and spirilla, elsewhere scarce. Later, during the day after, and the following days, this type of bacterium became more numerous and the colonies more abundant. Both in the fresh and stale sputa the disposition in groups of two, three, and four together, surrounded apparently by a capsule, was frequently observed, and rendered very distinct by staining with methyl violet.

The beaded forms, *s*, were observed in sputa immediately after expectoration, at times arranged side by side, sometimes interlacing, oftentimes longer than the specimens shown in the figure. Sometimes numerous examples were found in which the threads of the same minute articulations as form the type *s* run parallel. In the stale sputa, on the third to the fifth day, many of these threads took the appearance of long ribbons, by the formation of some connecting matter not only between the threads, but on all sides. This matter takes a pale violet colour, and evidently is connected with the production of the young forms of bacilli, after the style shown on the left and lower side of *n*. The limited space of this memoir will not permit of my giving an illustration, but the type shown in *b'*, Fig. 2, will give a very fair representation of it.

To return to the simple chain-like forms, these oftentimes so increase in length, and mat themselves together, that they present much more intricate forms than those shown in *u*, *u*, especially in stale specimens of sputa; the drawing was made from a fresh specimen, and magnified to 600 diameters. Such interwoven masses are found not only in whooping-cough, as we saw in the second section, but also in the sputa of phthisis, in cases of chronic laryngitis, and in inflammation of the lungs, especially a day or so after expectoration, and in warm weather.

I believe that these masses, of the type *u*, *u*, spring from the beaded forms *s*, for several times I have come across specimens just at the period of transition. Want of space prevents me from giving a drawing of these forms; so, too, many specimens of ribbon-like forms are not given for the same reason. These forms lead me to think that the bacilli of type *n*, on the right side of the plate, and below, are derived, as I have said, from the single articulations of the type *u*, *u*. There still remains the doubt whether the curved bacteria as at *o* in the lower part of the plate, coupled and surrounded by a cementing medium, can give origin to identical bacilli.

Again, in healthy sputa I have found short chains of ellipsoidal bacteria, *v*, among such masses as are shown in *u*, *u* ; such chains should be looked upon as the origin of the bacilli of type *n*, as seen on the left of the drawing. These bacteria contain, in fact, a close succession of similar bacilli, and a paler surrounding medium ; it should be observed that the chain, *v*, is drawn on a scale of 600 diameters, whereas the bacillus, *n*, is magnified to 2,500 diameters. Such transition is proved by the fact that I have found specimens which clearly showed the formation of the cementing medium among the individual bacteria, and ellipsoidal articulations when the sputum was again examined the following day. Figure *b'* will give an idea of such specimens, though taken from a bacillus of different character, viz., in a case of inflammation of the lungs.

The isolated bacilli, *v'*, *v'*, are distinctly the same as those of the type *q*, taking into account the difference of scale under which they are drawn. In *z* are shown many of these bacilli in long lines (2,500 diam.), tending to form a chain-like thread ; these appeared in the same sputa on the second day. They gradually increased in size, and by the fifth day, under a temperature of 28°C., took the form of distinct threads or tufts of *Leptothrix ;* thus we must take the forms *q*, *v'*, and *z*, to be but stages of one and the same bacillus, viz., *Leptothrix*.

The beaded forms, *x*, and the rounded bacteria of the type *y*, were also found in healthy sputa, and it is evident that the rounded members of such beaded forms are the same as the cocci in group *y*. The upper drawing at *x* deserves special attention, for it shows the identity of the oblong and rounded members in the specimen. We have here, in one and the same chain, an oblong member with three rounded bodies at each extremity, in little or nothing differing from those in Figs. *q* and *z*. This, as we have argued before, proves them to be identical. The figure *x'* proves the same thing, showing as it does two of the rounded forms, and three oblong ones at each extremity. The lower specimen in Fig. *x* shows identity with the bacteria in Fig. *r*, by the similarity of the middle portion. All these forms, therefore, *q*, *r*, *v'*, *x*, *x'*, *y*, and *z*, belong to one and the same species. We shall see that in like manner the bacilli *m*, *n*, *n'*, and therefore those of type *u*, *u*, and the beaded forms *s*, belong to the same species.

From this we must conclude that taking bacteria as the sole guide, the sputum of Whooping-cough does not differ from the sputum in health. In both cases the micro-organisms proceed from the many that habitually thrive in the mouth, and most probably in the nose as well. This conclusion is precisely that which Tschamer arrived at in 1870 from his first observations on Whooping-cough, as I stated in Section I.

### Bacteria and Bacilli found in Nasal Mucus.

Leaving for the present my observations on nasal mucus in Whooping-cough, I will make a few remarks on the microbes that infest the mucus in healthy subjects, for the germs of the bacteria found in the air-passages partially come from the mouth and partially, as I have said, from the nose.

The secretion from the nose in health consists principally of hyaline mucus, more or less dense, containing a number of granulated mucus corpuscles, which are endowed with a lively dancing motion as soon as they are subjected to any osmotic action. This motion is better observed after lightly staining, as their granules are rendered so much more distinct. But few vibratile epithelium cells are found, and these are generally of medium size, while some are very minute. They, specially at the beginning of an attack of coryza, show a rapid, persistent, vibratile motion; a large number of pavement cells of different sizes also appear. Some few red blood corpuscles are met with, and of course many foreign particles inhaled with the air. There is no trace of myelin.

Quite a large number of the dumb-bell-shaped bacteria are to be seen, as well as many of the types shown in *a*, *b*, *d*, and *e*, though larger than those figured in the plate. Above all the oblong forms, *q* and *z* are prevalent, some isolated, others in colonies or masses, many adhering to the epithelium pavement cells. Some of these oblong forms have a swollen extremity, resembling the *Vibrio rugula*. Other minute bacteria are usually present, more especially on the mucus corpuscles, and from these minute bacteria are derived those of the beaded type, *s*. There are, in moderate numbers, bacteria after the type of those shown in *l* (below), but without any apparent capsule, as also various diplococci with apparent capsules, as those shown in *l′*. These last were first noticed by Thost* and Lowenberg, in 1886, as will be seen from the Fig. 23 in the quoted work on Microscopical Diagnosis by Jaksch.†

Lastly, some few specimens of a rare bacterium, of the straight type, *p*, *p*, are found in couples, and now and then bacilli, quite small and very slender. In no single case did I notice in healthy mucus the *Streptothrix Forsteri*, but once found it in a rhinolitic concretion, having as a nucleus a cherry-stone, which had been buried in the right nasal fossa for a whole year.

## Bacteria and Bacilli found in the Sputa in Bronchitis.

In the clear mucus, during acute attacks of bronchitis, the bacteria are very numerous, accompanied by a few mucus corpuscles, together with some salivary corpuscles and buccal epithelia, a large number of pavement epithelium cells from the air-passages, as well as some few vibratile epithelium cells from the same, and in some instances by some red blood-corpuscles.

The bacteria found are those of the types *a*, *b*, *c*, *d*, *e*, *f*, *i*, *k*, *l*, *m*, *n*, *p*, *q*, *s*; to these may be added masses, as shown in *u*, *u*, of small slender bacilli, spirilli, and filaments of *Leptothrix*, etc. I must also draw attention to the fact—not noticed, I believe, by other observers—that the mucus corpuscles are at times invaded by minute diplococci, occasionally free, but sometimes apparently enveloped in a capsule. Such a fact is of no less importance than that which was held to be a characteristic of inflammation of the lungs—viz., the formation of numerous diplococci within mucus and pus corpuscles. Besides the imprisoned ones, others are to be seen with a semblance of a capsule, such as are shown at *f′*, *l′*, and *p′*.

* Thost, *Deutsche Medicinische Wochenschrift*, 1886, 12, 161. Lowenberg, *ibid.*, 446.

† Jaksch, *Manual* (quoted), p. 58, Fig. 23*c*.

BACTERIA AND BACILLI FOUND IN PNEUMONITIC SPUTA.

The same variety of types just enumerated are found in the sputa in cases of inflammation of the lungs, but the types with apparent capsule seem relatively to predominate.

The straight forms, as *p*, *p*, sometimes free, at times enclosed within a transparent envelope, and now and then covering and invading such buccal epithelium cells as are breaking up; their numbers increase day by day, especially in hot weather. The beaded forms, *s*, and the matted groups, *u*, *u*, are present.

The forms with clear surrounding matter vary in numbers in different cases, but never seem to predominate over the other forms. They oftentimes are mixed in the same group or mass with bacteria of different types. These, too, increase in numbers in stale sputa. We find also the forms, as in the lower part of Fig. *l* (biscuit-shaped), those as shown in *l′* on the right, the forms *e* and *f*, and that shown in *f′*, made up of four rounded bacteria or cocci strung together.

The apparent capsule is not merely the outcome of some optical illusion caused by contraction of the surrounding medium, leaving a vacant space, for such capsules are equally visible in specimens contained in newly added liquid medium, beyond the striæ of the mucus; and, moreover, some of the bacteria presenting such apparent capsules—those of type *f′*,—are endowed with a somewhat lively motion, and still carry with them the same appearance. But, still, there is not usually any distinct sign of a true capsular membrane or sac. It seems to me, therefore, that we have here simply a clear, transparent matter, not acted upon by any dyes, surrounding the bacteria, or else cilia of the most minute kind. This last supposition will explain the motion of these forms and the difficulty of staining the apparent capsule; but no one appears to have observed such cilia, even under the highest powers.*

* On this question of vibratile cilia, see, among others, Van-Tieghem, *Sur les prétendus cils des bactéries*, *Bulletin de la Soc. Botanique*, Paris, 1880. As regards the flagella of *Bacterium termo*, see Dallinger and Drysdale *On the existence of flagella in Bacterium termo*, *The Monthly Microscopical Journal*, 1875. Cornil and Afanasieff attributed the capsular appearance to the contraction of the medium (*Leçons à la Faculté*, etc., 1883—84); but Cornil seems

In my researches I have found that the capsular appearance of these bacteria is rendered more distinct by the use of methyl violet than by gentian violet. This may arise from the methyl violet staining more deeply and with a bluish tint both the bacteria and the surrounding mucus, thus showing up more distinctly the intervening and unstained zone.

All these capsular bacteria, but principally the smaller ones, invade the buccal epithelium cells in pneumonitic sputa, but in fresh specimens they are difficult to perceive within pus corpuscles.

In some pneumonitic sputa I have noticed the beaded forms, as in $x'$ ( × 1,250 diam.), where will be seen two rounded bacteria or articulations, with three others, elongated, at each extremity of the chain. These elongated forms in no way differ from those in $q$ and $x$ (above) and $z$; the difference of scale in the drawings should be borne in mind. This tends to establish still more the identity of the round and elongated forms.

In other pneumonitic sputa, examined at the same time, I have observed innumerable and most minute bacteria arranged in line among the striæ of the mucus as a string of beads. I believe them to be identical with the forms $e$, $f$, and $p$ (the straight specimens), but younger in growth.

Examining the sputa on the following day, which was a very warm one, I found these minute bacteria had increased in greater numbers than any of the other forms. Slender threads of growing bacilli, as $b'$, were also seen; among such chains as are seen in $c$ were noticed, with a greatly increased number of the straight bacteria, some of the type $p$. The invasion of the buccal epithelium cells by the bacteria was more and more evident.

In a case of inflammation of the lungs, the temperature being 30°C, some sputum was preserved in a closed glass tube, previously cleaned with sulphuric acid and rinsed in alcohol. On the fourth day the material turned quite black. I found this change was caused by the buccal epithelium cells turning to a clear brown,

to have modified his views. See, too, Dallinger on *The Cilia of Bacteria*; Carpenter, *The Microscope and its Revelations*, 7th ed., London, 1891, p. 587. The researches of Lœffler and others on the staining of these cilia are well known. As to the apparent capsule, the succeeding memoirs may shortly be referred to.—(F. V.)

and by the nuclei and granules taking a still much darker colour, which had also spread in a lesser degree to the other morphological contents. The sputum also contained a large number of red blood-corpuscles. It seems to me not at all improbable that some tannate or malate of iron had been formed by the mutual action of hæmatin and some tannic or malic acid, which could be accounted for by the patient having eaten some unripe grapes or seeds of pomegranate or similar fruits. The patient was a farm labourer.

I cannot believe any of these bacteria can have any specific character, whilst even the so-called pneumococci, with apparent capsule, are not exclusively found, either in pulmonary inflammation or pleurisy. It has certainly never as yet been proved that the germs of the bacteria with apparent capsule do not owe their origin to identical forms found usually in saliva ; and what is more urgent still is, that I have found these identical bacteria, as I mentioned before, within corpuscles of the bronchial mucus, in urine, and in the spermatic fluid. A youth suffering from spermatorrhœa, emitted in every defæcation some of this fluid, and on this being examined an abundance of spermatic filaments in lively motion was found, together with a few pus corpuscles and mucus. On this material being stained when fresh with methyl violet, I found present some of the dumb-bell bacteria, and not a few of the capsular bacteria of the type of those in *l'* and *f'*.

I am not pretending to bring this forward as a new discovery, for such apparently capsular bacteria and diplococci, morphologically identical with the so-called pneumococci, have been observed by others in many different substances. Talamon went so far as to say that *all those microbes that are found in bronchial mucus can assume a capsular appearance.**

It is well known, too, that the diplococci, whether capsular or not, which infest the blood, spleen, and liver in salivary septicæmia (an experimental disease produced by the subcutaneous inoculation of rabbits with pneumonitic sputa) cannot be differentiated from analogous forms found in the saliva.†

* *Comptes-rendus de l'Acad. de Médecine*, 1886.

† Some attribute the discovery of salivary septicæmia to Fraenkel ; but according to Cornil and Babes (*op. cit.*, p. 187), Klein appears to be the first to have inoculated rabbits with sputa from patients during inflammation of the lungs of the human subject.

As far back as 1885, we find that Fraenkel and Platonow observed this semblance of a capsule in the most varied forms of bacteria found in sputum,* and some few months later Sternberg also observed the capsular appearance in certain bacteria of the saliva.† Previously, Thost had described some *capsulated diplococci* and a *diplococcus identical with that of Friedländer*, which he observed in nasal mucus, both in the healthy state and in cases of Rhinitis and Ozæna.‡

More recently Netter noticed in the mouths of healthy persons *microbes identical with those observed in pulmonary inflammations and in salivary septicæmia.* §

Though it is true that the forms with capsule never seem to be so abundant in other cases as they are in cases of pneumonia and pleurisy; yet this may depend upon the peculiar condition of the secretion in the air passages and bronchial tubes in these diseases, which may favour their development and increase. ||

## Bacteria and Bacilli found in the Sputa from Pleurisy.

In genuine cases of pleurisy, where there is no collateral hyperæmia, the sputum is poor in the matter of morphological elements; it contains only a few pus corpuscles, and the red blood-corpuscles

* *Deutsche Medicinische Zeitung*, Feb., 1885.

† *American Journal of Medical Science*, July, 1885.

‡ *Berliner Klinische Wochenschrift*, 1884, No. 25, p. 388.

§ *Bulletin Médical*, May, 1887.

|| Hunter Mackenzie denies the specific character of the pneumococci, and quotes a case in which an unusually large number of these bacteria were found in the sputa of Hydrothorax. In a case of traumatic pneumonia, through the fracture of the fourth right rib, the sputa showed to me for many days an abundance of pneumococci. In cases of pneumonia supposed to be infectious, I have found these pneumococci in larger numbers during the latter period of the malady (as in the third week) than at its beginning; surely, on the supposition that such forms have a specific character, the contrary should take place. Lately, Sternberg (*The Medical Record*, March, 1889, Nos. 958—59) acknowledges their buccal origin, and attributes their lodging themselves within the lungs to a *peculiar disposition* arising from the very cause of the malady.

are frequently entirely absent. Where the fever is high and the difficulty in breathing acute, these capsular bacteria are even more numerous and larger than in pneumonia, as shown in *l′* on the left side of the plate and in *l* below; the quick and frequent inspirations carrying the germs from the nose and mouth will account for this. There are found in this sputa the dumb-bell form and others—even those of the type *p*, *p*, gathered often round the masses or tufts of *Leptothrix* when present.

On the third day, in a case of pleurisy with effusion, fresh sputum was treated with methyl violet, and was found to contain the bacillus drawn in Fig. 2, *n′* (× 2,500 diam.). The two ends of the bacillus are deeply stained and opaque, whilst the intermediate part is clear but granulated, and contains minute bacteria, some placed lengthways and others across the axis of the bacillus. This specimen may be taken as an example of transition of the younger forms, *n*, *n*, to the maturer forms, *m*, *m*, here evidently showing a stage of the transition. For in *n′* the two extremities are exactly similar to the forms *m*, *m*, whilst the middle part retains its likeness to that seen in *n* (below, on the left). There can be no doubt as to the identity of these two forms.

These bacilli of the type *n′*, and similar ones, surrounded a new formation of a tuft of *Leptothrix*, or really formed a part of it; and we may safely infer that the types *n′*, *n*, *m*, *u*, *s* are the same as *q*, *v′*, and *z*; and these, again, are the same as *x′*, *x*, *y*, and *r*.

Judging from the types shown in the plate and alluded to in the text, we may conclude that all may be reduced to two species: *Bacterium termo* and *Leptothrix*. We can refer the forms *a*, *b*, *c* (above), *d*, *e*, *f*, *f′*, *g*, *i*, *k*, *l*, *l′*, *p*, and *p′* to *Bacterium termo*, and the forms *b′*, *c* (below), *m*, *n*, *n′*, *o* (on the left), *q*, *r*, *s*, *u*, *v*, *v′*, *x*, *x′*, *y*, and *z* to *Leptothrix*.

Nor is it improbable that even these two forms are one and the same species. Cohn, Naegeli, De Bary, and others hold that *Bacterium termo*, *Leptothrix*, and the round bacterium (*Monas crepusculum*) are but three forms or stages of one and the same species. I am also of opinion that the forms known as *Vibrio* *rugula*, *Clostrium*, and *Rhabdomonas* are of the same species, being varieties of the same form of bacilli.*

BACTERIA AND BACILLI FOUND IN THE SPUTA IN PHTHISIS.

I can but merely touch on the matter of bacteria in phthisis, as it presents a field far too wide to be treated adequately in the short space of this memoir, and for the same reason I shall not be able to enter into a discussion of the methods of culture or inoculation. As my observations were only of a semiological character, and although I do not pretend to offer the results of a fully scientific research, I am of the firm opinion that the morbific character of bacteria cannot be fully established until the *natural history* of micro-organisms, in part at least, be first ascertained, and this, I believe, has yet to be done. This much is certain: that bacteriological research cannot be considered an infallible test in the diagnosis of disease, nor, without caution, can it be considered as pathognomonic in clinical medicine, until in daily practice it is found to tally with the other observations made during the course and termination of disease.

Having made this statement, and reserving for another place some statistics on the question, I will now proceed to offer an objection to the assumption of the existence of any tubercular or phthisical bacillus in sputa.

All the fervid supporters of this bacillus and its pathogenical power have arrived at the conclusion that its morphological characters are dubious and of little value, and that no bacillus can be assumed to be the tubercular bacillus, unless when stained with gentian violet it is not decolourised, although treated with a mixture of dilute nitric acid and alcohol. *Without this test, they say, no bacillus can be held to be the bacillus of tubercle, even if its form, length, and disposition be the same as that of the type described.*

* For the sake of brevity, I have omitted the distinguishing characters of the pneumococci (Friedländer's and Fraenkel's, etc.). Most likely, the differences observed are but accidental, and may arise, I think, from different degrees of staining imparted to this or that portion of each member of a microbe, to the point, side or connecting matter. Anyone can assure himself of the numberless forms, too many to describe here, found in any one mass of pneumococci.

These are the words of my esteemed friend, Prof. Primavera, a most strenuous upholder of this bacillus.*

The only distinguishing test between the bacillus of tubercle and other bacteria or bacillus in sputa is a relative difference in their staining power, or, in other words, a difference in their chemical value. I call it a chemical test, as the process of drying, the colourising with aniline dye, and the warming over the lamp must destroy all the vital properties of the bacillus.† Gentian violet is a basic stain, owing to the amount of oil of aniline it contains; it is readily soluble in alcohol, as are all other aniline dyes. The test, therefore, resolves itself into the question as to whether the affinity of the bacillus to retain the stain is stronger than that of the power of the acid and alcohol to remove it.

In other words, if the staining of the bacillus be only a chemical phenomenon, the decolourisation, too, must be of the same nature. So that the taking up and retaining the colour on the one hand, and parting with it on the other, will depend upon conditions altogether fortuitous and variable, and which must be of a purely chemical nature. Now, a purely chemical characteristic can hardly be elevated into a morphological or biological one, fit to determine a particular species of micro-organism. At the very most, it might be used as a criterion in diagnosis; but, even reduced to this, can we be sure that this criterion is not fallacious?

You may heat the preparation a little below or above the proper temperature; you may add a little too much or too little of the stain; you may apply it while the preparation is warm or cold; you may allow the dye to act for too long or too short a time; you may use a stain which is quite fresh or it may be old; you may allow the nitric acid *to act a second too long or too short;* you may, when washing the preparation in alcohol, allow the violet or green tint to be visible to the naked eye, or by the addition of more alcohol destroy the tint altogether. In each of these different suppositions you will necessarily obtain different results. In all probability, sometimes the results will be satisfactory in a

* Primavera, *Manuale di Chimica e Microscopia Clinica*, Napoli, 1887—88, p. 400.

† *Ibidem*, p. 399. Relating to the poisonous action of aniline oil on the above bacilli. See G. Sormani, *Nota al R. I. Lombardo*, 15 Dec., 1887.

practical sense; but when the microscopical examination is systematically deferred till the decolourisation with nitric acid and alcohol is complete to the naked eye, or, what is more important, until the alcohol used in washing is *completely colourless* as it drops from the slide, the reliable results will be considerably lessened and your faith greatly shaken in this distinguishing test.

It is some time since I noticed this difficulty, and only lately I happened to come across an observation of Jaksch to the same effect. This author recommends that the dilute nitric acid and alcohol should be allowed to act only long enough to change the violet tint to green. "*On ne saurait*," he adds, "*conseillier d'attendre que les préparations soient complétement decolorées, car, si on attend trop long temps, sous l'action énergique de l'acide, les bacilles (de Koch) finissent par se décolorer.*" *

Another source of error is the unequal distribution of the sputum on the slide; but I must now leave this question. Nor can I now give an account of the technique in use, but must reserve the matter for another time.

One thing, very evident from these bacteriological observations is, the very great affinity which the beaded forms of the bacilli in question have for gentian and methyl violet. But those who bear in mind the various grades of tint imparted to the bacteria by these stains will not be at all surprised, for we nearly always find that the more minute bacteria in sputa take the stain more tenaciously, with the exception of those more fully developed, which are enveloped in some sort of cuticle (as we saw in the case of the forms *i* and *k*); in such the opposite takes place—the larger and full-grown specimens are the more strongly tinted. Now, the individual bacterium or articulation that forms the beaded type, *s*, is the smallest met with in sputa, and it is not unlikely that

* Jaksch, *Manual*, p. 73. It is well to notice that the Gratz' Professor, a strong upholder of the value of this bacillus in sputa in diagnosis, uses methyl violet. Hunter Mackenzie does not believe in the selective power of this bacillus for gentian violet, preferring fuchsine; but this even disappears *si le bain d'acid nitrique est trop prolongé* (more than 30 secs.) (*V.*, *loc. cit.*, *p.* 141). Lately, carbolated fuchsine has been recommended (*To Stain Tubercle Bacilli*, in *The Journal of Microscopy and Natural Science*, edited by A. Allen, July, 1889, pp. 165—66), and which answers well in practice.

for this reason alone, or on account of the enveloping cuticle, and not through any biological faculty, they are able to take up the dyes more energetically.

But there is still more to be said on this subject. This power of retaining the dye does not exclusively belong to the bacillus of Koch, as I have satisfactorily proved in my own experience.

In the first place, it is not an unusual thing, in the examination of phthisical sputa, which has been stained when dry with gentian violet and decolourised with nitric acid and alcohol, to find a small foreign body, such as a shred of minute membrane, or frustule or filament of vegetable origin, or some epithelium cell which still retains the blue tint. Then if such foreign body is covered with, or retains any bacteria, these will be found unbleached, though they are of a type altogether different from that of Koch's bacillus. These bacteria are extremely minute, yet after the type of those in *l'* and *p'*, usually found in the sputa under consideration, not only when fresh, but even after treatment with potash, as I shall presently show.

In the second place, we not infrequently find in such dry preparations, treated with gentian violet and then decolourised, colonies of minute capsular diplococci, of the types *f'*, *l'*, and *p'*, sometimes singly, or two, three, or four together in one capsule. These colonies alone retain a distinct, though faint, tint or shade of colour, which allows the individual members to be made out when mounted in a clear, refractive mounting medium (oil of cloves or glycerine); while all the other forms, not including those of the beaded type, *s* (Koch's bacilli), are bleached, and consequently invisible.

From this it is evident, not only that many bacteria of various types retain the gentian violet stain, but even do so at times in higher degree than the so-called tubercle bacillus. I have observed many striking examples of this in pneumonia. The specimens, which were from sputum extraordinarily rich in pneumococcus, had been dried and stained with gentian violet. Anybody can verify their resisting power *to a second attempt to bleach them* with the usual dilute nitric acid and alcohol mixture, though the retained tint is faint.

Nevertheless, it is not my intention either to impugn or affirm the existence of the bacillus of tuberculosis, but simply to raise a reasonable doubt on this most important question of clinical microscopy, which is still *sub judice*. The judges of the matter in the first case may be the band of technical investigators, but the ultimate and definite decision must be left to clinical practitioners.

One of the most striking arguments to support the idea of infection or contagion by bacteria is the diffusion and reproduction of these micro-organisms through the tissues in different parts of the body. Although this question only touches us indirectly, I would observe that all living material, whether healthy or in a state of decomposition, is liable to this whenever the parts are sufficiently attenuated to allow the particles to pass from their first nidus to parts more or less deep, so we must not be surprised to find in autopsies that the tissues have been invaded by bacteria, and especially by those that thrived at the seat of the disease. There is no reason for urging this as a proof of the morbigenous power of the organisms in question. In fact, the very opposite opinion that such *bacteria are harmless in general* can offer a very simple and plain explanation from the *alteration of the affected parts*. It is but a general law, which holds good both in a healthy and a morbid state, that *the products of disassimilation* in the higher organisms become, *ipso facto*, *the source of assimilation* in micro-organisms; and the reason is this, that bacteria deprived of chlorophyll are incapable of decomposing the anhydrous carbonic acid of the atmosphere, and their growth is carried on at the expense of higher organisms. This law accounts for the diffusion and the increase of micro-organisms wherever particles of matter are found in disintegration or in any part removed from vital influence, the sovereign power in higher organisms. In all cases where such alteration of living matter is in progress, and where the bacteria or their germs are not excluded by special and exceptional means, we not only *find micro-organisms, but they are necessarily there, and cannot by any possibility be absent.*

Further remarks on this point will be found in a previous communication of mine on *Relapsing Fever*, where I have brought forward other objections to the supposed power of bacteria in communicating and spreading disease.*

I think, moreover, that the relative predominating number of the beaded bacilli, *s*, can be explained without having recourse to the assumption of their power to originate disease. During the chronic process of disintegration in the pulmonary substance, the morbid products will naturally be more frequently, and for longer intervals remain, lodged in cavities, not being carried to other points by the currents of air in respiration. This quiescent state tends to the further decomposition of the morbid secretions, and we have seen in the second section (*Sputum in Pneumonia*, July 31), and in the fourth section (*Bacteria*, *etc.*, *in Healthy Sputa*), that in sputa undergoing decomposition the more minute forms of bacteria are predominant. In the second place, this quiescence and immobility of the menstruum, or nourishing medium, must necessarily favour the juxtaposition and formation of the single bodies in lines or threads, then to their multiplication and increase in linear series, and eventually to the formation of beaded strings, and from that to rods or filaments. This can be verified in all kinds of sputa (not excluding healthy sputum) whenever they have been closely kept in vessels, and day by day the beaded bacilli may be seen to thrive and multiply, together with the interwoven growths of bacteria.

Returning to my own observations, I maintain that the bacteria found in phthisical sputa, which has been stained when fresh, does not substantially differ from that found in other sputa beyond the numerical predominance of this or that type. In some the form *s* may abound, and in other specimens it may scarcely be seen. The interwoven forms—as *u*, *u*—may at times be observed, more especially when the larynx is affected, probably on account of the ease with which morbid secretions can be lodged for some time in the folds or recesses of that organ. In dry preparations

* *Sopra un caso di febbre ricorrente e sul riscontro degli spirilli nel sangue*, *Atti R. Accad. Med. Chir. Napoli*, Vol. XXXVI., 1883. I would remark in this matter that Ebstein gives an account (*Berliner Klinische Wochenschrift*, 31, 1887) of a case of *chronic relapsing fever* with nine relapses and a total duration of 211 days, a case analogous to one described by myself four years before, but in which there were six relapses in a course of 94 days.

the beaded forms *s* seldom resist the decolourising action of nitric acid and alcohol. But any conclusion arrived at in this way, as to the nature of the bacteria, is but a doubtful one. Any two observers may easily arrive by it at very different conclusions; hence, unfortunately, such conclusions as may be arrived at cannot be relied upon in judging of the state of a patient. I am of opinion that of the bacilli in sputa, those showing a reticulated structure (they are unbleached by the usual reagent) are a much better guide in the diagnosis of phthisis. If any be taken as such, I, however, take them to be simply an unstudied form of *Leptothrix*.

In this sputum the dumb-bell bacteria are found in profusion, unchanged in form, and groups and colonies of considerable size, even in the sediment from sputum which has been treated with an emulsion of potash. Both the free and capsular forms of bacteria of the type *p*, *p* are also very abundant.

Those apparently capsular bacteria of the type *p'* are quite visible in the sediment from emulsionised masses of sputa, even when they are mixed with other minute forms of bacteria or diplococci, surrounded with the same kind of clear capsule, and which, though of smaller dimensions, resemble the forms of the types *p'*, *f'*, and *l'*.

It may be well to state here that, after having treated and heated the sputa with potash, it is my custom, when searching for the elastic pulmonary fibres, to allow the sediment to settle in a conical glass, and by shaking and twisting the glass to hasten the descent of the tufts that might settle on the sides. When the sediment has been well deposited (which may take some two hours), I decant the clear, supernatant liquid, and add about as much distilled water, stirring the whole with a glass rod to break up the sediment. In this way it is thoroughly washed from all the numerous granular particles and oily globules that would otherwise spoil the preparation. If one washing is not sufficient, I use another.

Preparations from these deposits are excellently stained, when fresh, with gentian violet or methyl violet, rendering the beaded types *s*, with the other bacteria and diplococci, both large and small (spoken of above), very distinct, as well as the apparent capsular forms. Very often the above beaded forms and bacteria of other types are mixed together in one group or mass.*

In the article on Sputa in Whooping-cough (see the first chapter of this Section), I spoke of the bacillus *c*, *c*, of which I found some few specimens in this class of sputa.

Summing up what I have hitherto observed, my opinion is that the character of the beaded bacillus of the type *s* is altogether accidental; while the contrary opinion respecting their specific and pathogenic character is confronted with two very grave objections. Firstly, that these beaded forms are found to exist in other kinds of sputum, including that of healthy subjects. To this objection it may be answered that the beaded forms are spurious when they do not resist the decolourising action of the nitric acid and alcohol. And secondly (and which is a more serious objection), these beaded forms, *also from phthisical sputum*, abound, not only surrounding, but actually within, the *very body of the buccal epithelium cells* in the process of breaking up. Some of these cells are literally packed with the bacilli. This can hardly be explained except by granting that the origin of such bacilli is to be found in the mouth.†

Respecting the diagnostic value of bacteria in the examination of any kind of sputa, I maintain that it is quite impossible to

* This method of examination for Koch's bacilli in sputa treated with an emulsion of potash is of my own devising. I find that Biedert uses a modification of this method when staining and examining the sputa in its natural state. He adds a little fresh egg albumen, and then stains a portion of the albuminous deposit. (*Journal of Micro. and Nat. Sci.*, N.S., Vol. II., p. 166, July, 1889, *loc. cit.*)

† In a *resume* of a lecture from Sternberg at Albany, written in February, 1889 (four months after my communicating the results of my researches to the Royal Academy of Naples), I find the following quotation, which, though sustaining other views, seems to predict the fact, already observed by me, that Koch's bacilli might be found in buccal epithelium cells, even under normal conditions :—" It is quite probable, in the present state of our knowledge, that the tubercular bacillus may live in the buccal mucous membrane, in that of the nose, or even in the larger bronchi of those persons living in crowded districts, and that it then attacks the pulmonary tissue when this last is predisposed, in an unhealthy condition, or so injured as to present a suitable medium for its development."—*The Med. Record*, 1889, Nos. 958—959.

ascertain from *any bacterioscopic investigation* whether the sputa is from a healthy or from a diseased subject; or, under the second hypothesis, what is the nature of the disease? There may be exceptional cases—sometimes in tuberculosis, pneumonia, and pleurisy—to this general rule, as in such cases of tuberculosis where the beaded forms, *s*, by combination, resist the decolouring medium, or are accompanied by reticulated bacilli; and in those cases of pneumonia and pleurisy, in which the forms of bacteria or capsulated diplococci are in such quantities as to outnumber all other bacteria present. In all other cases, if any certain conclusion is arrived at, it will never be from merely bacterioscopic examination, but from other characters of the sputum.

§ V.

## OBSERVATIONS AND CONSIDERATIONS ON THE FUNGI.

As I before stated, I have observed two kinds of fungi in sputa of Whooping-cough. One, the most conspicuous, was met with in three cases out of the fifteen. In the first of these cases, it was found in all the sputa examined up to the end of the attack. It was not found at all in the pulmonitic sputa of the patient after his recovery from the Whooping-cough. The other fungus, which was much smaller, was observed only in one case; but it might have escaped my notice through want of time and patience in searching for it.

The larger fungus was formed of very distinct tufts of filaments, freely germinating in the sputa from the day after its collection. In Fig. 3, *d*, is shown the middle portion of one of the tufts (×800 diam.). It was stained a pale blue by gentian violet; but the single cells are drawn somewhat more oval in the figure than they were in the specimen. The *mother* cell of the tuft is seen at *e*; on the left will be noticed a smaller cell, from which arise the base of two filaments, partly seen in *f* and *g*. Between the cell and the base of the filaments there was a small granular mass more deeply stained than the rest. On the right of the *mother-cell* two filaments branched out directly, with the granular mass at the base, as in *h* and *i*. These filaments or threads of the mycelium, little by little, become more slender, and were more deeply stained at the extremities when they were treated with gentian violet, and even more distinctly so when treated with carmine. This is observed in all such tufts, and arises from the germinal matter in activity being so much denser at their extremities.

As the second fungus does not present any striking peculiarities, I need not dwell upon its filaments.

Many vesicular gemmules, sacculi, or sporidia were given off, as I have said, from these larger tufts of the type *d*, and many were observed quite detached, sometimes isolated, sometimes united in small groups. Two of these are shown in *b*, *b*, as they appeared in an ordinary preparation, stained with gentian violet, and magnified 2,500 diameters. To better observe the interior structure, I stained a suitable specimen of sputum with Beale's carmine. My method was, first, to add a drop of diluted acetic acid to a particle of the sputum, and then place it in a watch-glass containing the carmine stain, taking it out the moment it appears to be stained sufficiently, otherwise the tint becomes much too diffuse. One of these gemmules is shown in *c*,* stained in this manner with carmine under the same power as *b*. It will be seen to present a lightly stained exterior layer, and an interior one containing granules more deeply stained. These granules are more vividly stained within the narrow and pointed neck, where the two layers or coats seem to run into one another. Probably a new growth of gemmules begins at this point, and at the same time new tufts are reproduced. The contents seem to be a homogeneous matter, in which are suspended some masses of germinal matter and granules darker than itself.

To obtain the fructification of the fungi in qnestion, I prepared a culture on the inner surface of a fresh piece of lemon peel by placing on it a little of the mucus which was rich in the tufts, and enclosing it in a glass vessel, which was then placed in a dark cupboard. Another culture was tried on potato and flour paste without any result. Perhaps these substances dried up too quickly at a temperature of 22° C. The first culture was examined every morning. On the fourth day I found it covered with mould in greenish patches, from which arose little dark heads, quite

* This figure should be carmine, but has been tinted violet for purposes of illustration.

visible under a single lens. The greenish patches were the smaller fungus; the small dark heads the larger one. After two more days' growth I sliced off the aërial hyphæ with a scalpel, placed it in a drop of glycerine on a glass slide, and applied a thin cover glass supported by two slips of paper, to prevent the little heads from being crushed.

The fructification of the larger fungus—which, so far as I can judge, belongs to the genus *Mucor*—is shown in its natural state, *k* and *k'*, being mounted in glycerine and magnified to only 140 diam. The reproduction of the drawing of *k* is somewhat imperfect, owing to the difficulty of representing exactly the degree of transparency at different points and the tone of the natural colour.

The hyphæ of this fungus are not septate. They are slightly granular towards the extremities, clear and transparent elsewhere, and terminate in a rounded head, smooth and comparatively large, as shown in *k'*. This head is entirely covered by a layer of rather dark spores closely packed together. Fig. *k'* shows some of the spores still attached, while the rest have fallen off. The Fig. *k* shows the layer of spores intact and enclosed by a thin, darkish envelope, which barely allows the spores to be seen at all. It is somewhat more easy to make them out at the edges, and still more so at the point of attachment to the stem, where the envelope seems to be thinner. The detached spores are shown in *l* ( × 1,000 diam.), somewhat shrunken through exosmosis under the action of glycerine. Probably the fungus noticed by Tschamer, as I mentioned in the first paragraph, belongs to this species.*

The fructification of the more slender fungus is very much smaller, and shown in *n*, *o*, and *p*. The preparation was mounted in the same medium, magnified to 800 diameters. The hyphæ of this fungus are not septate, and give rise at their extremities, which are swollen, to a series of *sterigmata* or *ramules*, bearing

* I refer this fungus to the genus *Mucor (stolonifer?)* from the following description given by J. Payer :—"*Mucor*, *Micheli*. *Flocci erecti*, *simplices*, *continui*, *apice thecam subglobosam*, *cyatho laceram gerentes*. *Columna cylindrica vel ovata*. *Sporæ simplices*," in his *Botanique Cryptogamique ou Hist. des Familles Natur. des Plantes Infér.*, Paris, 1868, p. 85. As regards the vesicular gemmules of *Mucor*, see Cooke, *Les Champignons*, *sous la direction de M. J. Berkeley*, Paris, 1875, pp. 51, 52.

an erect string of spores. The fructification in *n* is scarce, there being but four ramules; in *o* it is abundant, although I should add that the specimen shown at *o* had lost many of the spores in the upper part of the branches, for in many other specimens the fan-like pencils are three or four times as long, contracting as they rise and ending in a plain horizontal section, as is often observed in *Aspergillus nigricans*. In the specimen shown in Fig. *p*, the fructification is covered with a dark, opaque veiling, through which only the marginal spores can be seen ; but in many speci-cimens the veil only partially envelopes the spores, sometimes at the lower parts and sometimes longitudinally. So it would seem that the various phases of fructification denote no specific difference, but are those of one and the same species.

This fungus may be *Aspergillus fumigatus* or a similar species, or perhaps it belongs to the genus *Rhodocephalus*.*

Probably the fungi found by Ransome in the breath of patients with Whooping-cough (see first section) were the same as the above. But I hardly think he can be right in describing it as *Penicillium*, as the hyphæ of *Penicillium*, as well as their ramules, are septated, whereas those of this fungus are continuous.

I observed in Section II. (Sputa of May 10th, *c*) that the smaller fungus, after some days, gave the sputum a greenish colour. I may here add that even on the fourth or fifth day this colour may be observed by transmitted light. If the sputum be preserved in a glass tube, the formation of cloudy patches of a light green colour will be easily noticed. Later the whole mass becomes green by reflected light. I tried to discover whether or no the development of these fungi correspond with any chemical change in the sputum, and found that this was not the case. The

* "*Aspergillus, Micheli. Receptaculum floccosum erectum, continuum, simplex, apice in capitulum inflatum. Flocci sporarum capitulum tegentes.—Rhodocephalus, Corda. Receptaculum floccosum erectum, continuum, non septatum, apice in ramulos simplices, capitulum formantes divisum. Flocci sporarum apicibus ramulorum singulatim innati.*"—Payer, *loc. cit.*, pp. 70, 71. In these definitions the hyphæ are sometimes called *flocci*, and at other times called *receptaculum*. *Flocci sporarum* are the moniliform strings of spores that rise from the top of the hyphæ or its ramules, or from the *columna*. For *Rhodocephalus*, see Fig. 303 of the same author, showing the fructification of *Rhodocephalus candidus*.

sputum in Whooping-cough, when quite fresh, gives a neutral or very slightly alkaline reaction; and up to the seventh day the reaction remains the same, notwithstanding the visible formation of these greenish patches and the increase of accompanying tufts in the preparation.

There is nothing new in this matter of specimens of vegetable growth in the air-passages. Nowak says "that micromycetes (specially the genera *Aspergillus* and *Mucor*) are frequently found in the air-passages of birds suffering from respiratory diseases."* And Perroncito, speaking of *Aspergillus fumigatus*, says that it has been found in the human lungs (Virchow and Pagenstecher) and in the air-sacs of *Otis tarda*, producing these pneumonitic nodules (*Pneumomicosi aspergilliana*). Passing over the Aspergilli that are found living in the auditory canal, the same writer quotes cases of *Aspergillus nigricans*, found by Robin in the air-sacs of a pheasant, and by Generali and others in other birds; of *Aspergillus virens*, also found in the air-sacs of birds; of *Aspergillus candidus*, found in the air-sacs of *Pyrrhula vulgaris*; and of *Aspergillus glaucus* in the air-passages of crows and other birds.†

The sputum that furnished the above specimens of fungi was kept in a tightly closed glass vessel until it was completely dried up. On the dried film at the bottom of the vessel there was no aërial vegetation, but on the sides there were growths, white in colour, of the smaller fungus, as shown in *n*, *o*, and *p*. I suppose that these growths were caused by particles of sputum reaching the sides whilst tipping the glass and extracting portions for examination.

In no way do I attribute any pathological importance or specific power to these two species of fungus which are found in the sputum in Whooping-cough. Their forms are diffused in profusion in the air, and in the cases mentioned they may have accidentally found their way into the sputum, as they were met with in these few cases only. I may remark that microscopic fungi

* Nowak, *Le malattie infettive, etc., trad. di Vestea*, Naples, 1884, p. 10. Payer, *loc. cit.*, p. 60. Beale, *loc. cit.*, p. 500. G. H. Mackenzie, Figs. 5 and 24. Jaksch, Figs. 30 and 31.

† Perroncito, *I parassiti dell'uomo e degli animali utili*, Milan, 1882, pp. 78, 79.

found living in sputum is not a new discovery. I have often found that when pneumonitic sputum has been kept for some days in closed tubes, a rather dark scum of mould rises to the surface; this is not to be confused with the diffused darkening spoken of in the fourth paragraph, for under the microscope it proved to be *Penicillium*. Other tufts are at times found in phthisical sputum and in the sputum of persons suffering from buccal disorders, or even when wearing artificial teeth. I have obtained a fungus of the type *k*, and observed its fructification, together with specimens of *Penicillium glaucum* from sputa of persons suffering from typhoid fever. These last are very common fungi, thriving and fructifying together on common flour-paste.

As regards the spores from the cistern water, of which I spoke in the third paragraph, and of which I give a drawing of a specimen when germinating in Fig. 3, *a*. I am in doubt whether it belongs to the genus *Hysterographium*, of the *Hysteriaceæ* family, or to the genus *Septosporium* of the *Botrytideæ*.* These are diffused everywhere in the country, even on vine-tendrils. So far, I have never observed them in water laid on in towns, but they are of frequent occurrence on wall-papers in moist situations or where the air is confined.

Naturally, I must leave the exact determination of the three species of fungi, shown in Fig. 3, to more competent micologists.

* *Hysterographium, Corda. Conceptaculum simplex, sessile, rima longitudinali dehiscens. Thecæ paraphysibus mixtæ, tubulosæ. Sporæ septatæ.*"—Payer, *loc. cit.*, p. 97. Compare with Fig. 433 of the same author, showing the spores of *Hysterographium elongatum*:—"*Septosporium, Corda. Flocci erecti, ramosi, pauco septati. Sporæ cellulosæ, pedicellatæ*."—*Ibidem*, p. 76. Compare with Fig. 340 of the same author, showing *Septosporium bulbotrichum*. The spores of the genus *Hendersonia* are longer and narrower."—*Ibidem*, p. 83. Compare with Fig. 49, Cooke, *loc. cit.*, p. 112.

§ VI.

## HINTS ON THE PREPARATION AND STAINING OF SPECIMENS.

Leaving to another time the determination of the source and seat of the production of substances ejected from the mouth, so that other substances—as mucus coming from the nose, or merely from the throat, or fragments of food—may not be mistaken for expectorations. I will now offer a few remarks as to my method of research.

When about to examine a quantity of sputum, I collect it when possible on a plate, spittoon, or glass, or I receive it on a clean cloth, from which it is removed by means of a spatula to any of the above vessels. At other times it was found to be sufficient to collect a little in a glass tube, thoroughly cleaned with sulphuric acid and washed in alcohol.

In the examination of sputa in the natural state, it will be sufficient, if a portion be taken about the size of a seed of millet, no diluent should be used except a little solution of sodic chloride, if the sputum is proved to be too dense and tenacious. In staining, even a smaller portion may be taken when the consistency will allow of it. This small portion may be taken up with a curved needle, or if too liquid a similar quantity may be transferred to a glass slide by means of a pipette or dropping-tube.

Having placed the portion upon the slide, and, if necessary, spread it with suitable needles, a drop of the stain is applied from the end of a glass rod, unless the stain is carmine, in which case another method should be followed, as below. As soon as the film of the sputum is seen to be sufficiently stained, it must be washed, by allowing drops of distilled water to fall on it, and these drops are made to run off by tilting the slide. This must be done until all granulated deposit from the dye be removed. The specimen is then again spread by needles, reducing it to a thin uniform film, so to say, a little less in area than the covering-glass to be used. A cover-glass should be used of known thickness, and should be gently pressed down by degrees at different points with the handle of a mounting glass rod well cleaned, so that the film of the specimen be rendered as thin as possible and the cover-glass made to adhere as firmly as possible, and air-bubbles squeezed out at the same time. Without these precautions, the cover-glass may attach itself to the front lens of the objective when the immersion oil is used, and so render any good observation almost impossible, on account of the difficulty of focussing and of the displacement of the specimen under the cover-glass, which at times may be even lifted up from the slide by its adhesion to the objective.

After the preparation has been examined under a dry lens, the condenser is adjusted and the immersion lens substituted, each portion of the specimen being successively examined. If during the examination (generally a slow operation in these thin layers of mucous matter) there are signs of the edges of the specimen becoming dry, a small drop of distilled water, very slightly tinted with the dye used, is allowed to fall on the opposite side close to the cover-glass, and by means of a needle drawn up to the cover and round its edge for a short distance, to render the flow of the water between the glasses easier.

As regards the dyes I have used, I will briefly offer the following notes :—

*Gentian Violet.*—I generally prefer gentian violet when examining sputa of Whooping-cough for the reason that it is a double stain ; the filaments, particles, and granules of myelin appearing a more or less deep blue, the true morphological elements and the bacteria being stained violet. This double staining affords great ease in observations, and moreover renders most distinct the myriads of the minutest granules of myelin, which would otherwise be passed over or mistaken for amorphous granulated matter or for micrococci. These granules are mostly to be found, often in lively motion, in those clear spaces (or little gulfs, as they might be called) formed by the liquid medium flowing, as it will do in these preparations, backwards and forwards, within the specimen; or else they will be found around the edges of the preparation, where the granules and particles of myelin are carried by the tiny centrifugal currents. So also the larger particles and masses are well marked, the gentian violet rendering them less refractive ; but it does not prevent the internal striæ from being quite visible.

If the staining has been carried too far, the tint of the myelin, after four or five hours, changes from blue to violet. Moreover, the excessive stain is decomposed, and runs together in smaller or larger drops of a deep blue colour, and at last they surround and absorb the myelin particles, which then begin to disappear, remaining only visible where the staining was more feeble. So it is important to avoid staining too deeply, for this would render the preparation quite useless after a few hours. As I have stated before, the preparation should be carefully washed with distilled water after staining.

*Solution of Iodine in Potassic Iodide.*—This stain, either applied as above or simply allowed to run into the preparation by capillarity, gives the myelin a very brilliant yellow colour, making it quite easy to recognise this substance. Pus corpuscles, bacteria, and epithelium cells are either stained very feebly, or not at all, with the exception of the ellipsoidal or alveolar epithelium cells, which then show distinctly their myelin granules. This stain has one drawback: it does not colour the thin granules and filaments of myelin, which are at best feebly stained by gentian violet.

*Picric Acid.*—The saturated solution of this acid in water imparts a pale yellow tint to myelin, but renders the internal striæ rather indistinct; bacteria, pus corpuscles, and epithelium cells are stained to a deeper yellow. It is a useful stain, the smallest particles of myelin becoming visible, as it diminishes their excessive refrangibility, and when the simple round diaphragm is used the molecular motion is most distinctly seen with it. The granules of myelin are then observed as minute points, somewhat darkened when out of focus, but quite brilliant when *within* the true focus. But in a few hours the myelin begins to undergo a slow decomposition; it is reduced at first to mere faded fringes, somewhat akin to those produced with oleic acid and liquid ammonia, and these eventually totally disappear.

*Methyl Violet.*—This stain renders myelin more opaque, and sooner or later dissolves it into a homogeneous mass of a pale blue colour. This mass adheres to any corpuscular bodies near it, and takes many fantastic shapes. I have already spoken of the advantages of methyl violet as a means of distinguishing the apparent capsule of certain bacteria in pulmonitis and pleurisy.

*Fuchsine.*—This stain, either diluted or used in small proportions, is very effectual in showing up the smaller granules of myelin, as it increases their refrangibility very considerably. At the same time, this increase makes it necessary to moderate the light as much as possible, and then the feeble light will not allow the higher eye-pieces to be used, without which the very minute granules of myelin are not seen. Fuchsine, too, unfortunately is apt to render somewhat indistinct the internal striæ of the particles and masses of myelin.

*Aniline Blue.*—This stain is inferior to the foregoing as regards making out either the different forms of myelin and bacteria from the fresh sputum, and so I have only used it experimentally.

*Carmine.*—This is most useful to determine the internal disposition of parts and the cortical layers of the vesicular gemmæ of the tuft, *d*, Fig. 3. As I observed in a preceding paragraph, it does not stain any form of myelin, even when portions of sputum are left in it for several weeks. I have always used Beale's carmine stain. This solution, on account of the glycerine, renders almost invisible the myelin granules, and even the particles and masses of myelin are barely seen in it. This may be because the refraction index of myelin and glycerine are about the same value, or at least because the difference is too small for distinct vision.*

## Resume.

From the foregoing remarks I may briefly conclude—

I.—In severe cases of whooping-cough, during the period of convulsions, myelin is found in the sputum in the greatest abundance, and bears no relation with the number of ellipsoidal epithe-

* The correspondence between the myelin found in sputa with the myelin of the nerve-fibres should be noticed. In staining the nerve-fibres with carmine the medullary sheath is not stained, while the cylinder axis is stained a full red (Frey, *Man di Tecn. Micr.*, Naples, 1873, pp. 236 and 245. Ranvier, *loc. cit.*, pp. 97, 98 and pp. 722—724). I have recently tried all the above methods of staining on myelin flowing from nerve-fibres and on preparations of nerves from animals recently slaughtered, with the result that I find that myelin in sputa behaves in exactly the same way as myelin of the nerves. This is surprising, especially when picric acid is used, for a preparation of healthy sputum mixed with myelin from nerve-fibres cannot be distinguished from the sputum of whooping-cough. I hope to treat this matter on another occasion.

lium cells, nor with any inflammatory or febrile symptoms, but solely with the intensity of nervous excitement. It certainly cannot be imputed to any idiosyncrasy of individual patients.

II.—That though there is a formation and elimination, daily, of small quantities of myelin from the air-passages in the state of health, in no other state or affection of these passages is such a remarkable quantity ever observed as in cases of whooping-cough, if we except the one case of hæmoptysis mentioned by Beale.

III.—The extraordinary abundance of myelin may account for the viscosity and transparency of the sputa of whooping-cough, and also for the difficulty of expectoration, and may in many cases serve as a guide in diagnosis.

IV.—That if the facts I have stated in this memoir are confirmed by further observations, and the nervous phenomena of whooping-cough are proved to be connected with the very great abundance of myelin in the sputa in other epidemics and in other countries; this constant connection may open a new view of the pathogenesis and etiology of whooping-cough, or may at least induce others to undertake similar researches on the nature of expired air, on the structure or condition of the medullary sheath of those nerve-fibres that secrete myelin, and which are distributed throughout the mucous membrane of the air-passages, and which lie as a network just beneath the layer of epithelium at all points where the mucous membrane extends.

V.—That the bacteria generally found in sputa, and particularly in the sputa of whooping-cough, arise from germs, in all probability, lodged in the nose or mouth; so that, until the contrary is proved by definite facts, their presence in sputa should be held to be in no way connected with the cause of the disease.

VI.—That the bacteria—though of many and divers forms—can be reduced to two types, of which they seem to be only so many different forms or phases of transition. One group can be identified with *Bacterium termo*, the other with *Leptothrix*, and probably these two belong in reality to one species.

VII.—That in any case, *by simply relying upon the forms of the bacteria found in sputa*, it is impossible to judge with any certainty at all whether the sputum examined be from a person in health or disease, and on my second supposition, to determine the nature of the disease, except in cases of tuberculosis, in which Koch's bacilli, chiefly of a cancellated appearance, are not bleached under the usual test, and in those cases of pulmonitis and pleuritis, where those apparently capsular forms are in the greatest abundance, forms which nevertheless are met with in other sputa, in acute bronchitis especially, they are found in large number within the mucus corpuscles, and they are also found in urine and the semen. But even in these excepted cases of tuberculosis, pulmonitis, and pleuritis, *the bacterioscopical examination* alone cannot be assumed to be a positive and certain guide in diagnosis.

VIII.—That any conclusion arrived at in a diagnosis from the discovery of Koch's bacilli in phthisical sputum must, in many cases, be doubtful. For even admitting that such a bacillus is the cause of tuberculosis, which has yet to be proved, it has not, in the first place, any special morphological characteristics; and, in the second place, it is decolourised by the action of nitric acid and alcohol, though perhaps in a less degree than are the other bacilli and bacteria of sputa, if the decolourising test acts too far.

IX.—That fungi are found in whooping-cough sputa only in a few cases; judging by the germination of the species observed, one appears to belong to the genus *Mucor*, and the other to the genus *Aspergillus*; in all probability both of these are inhaled from the air.

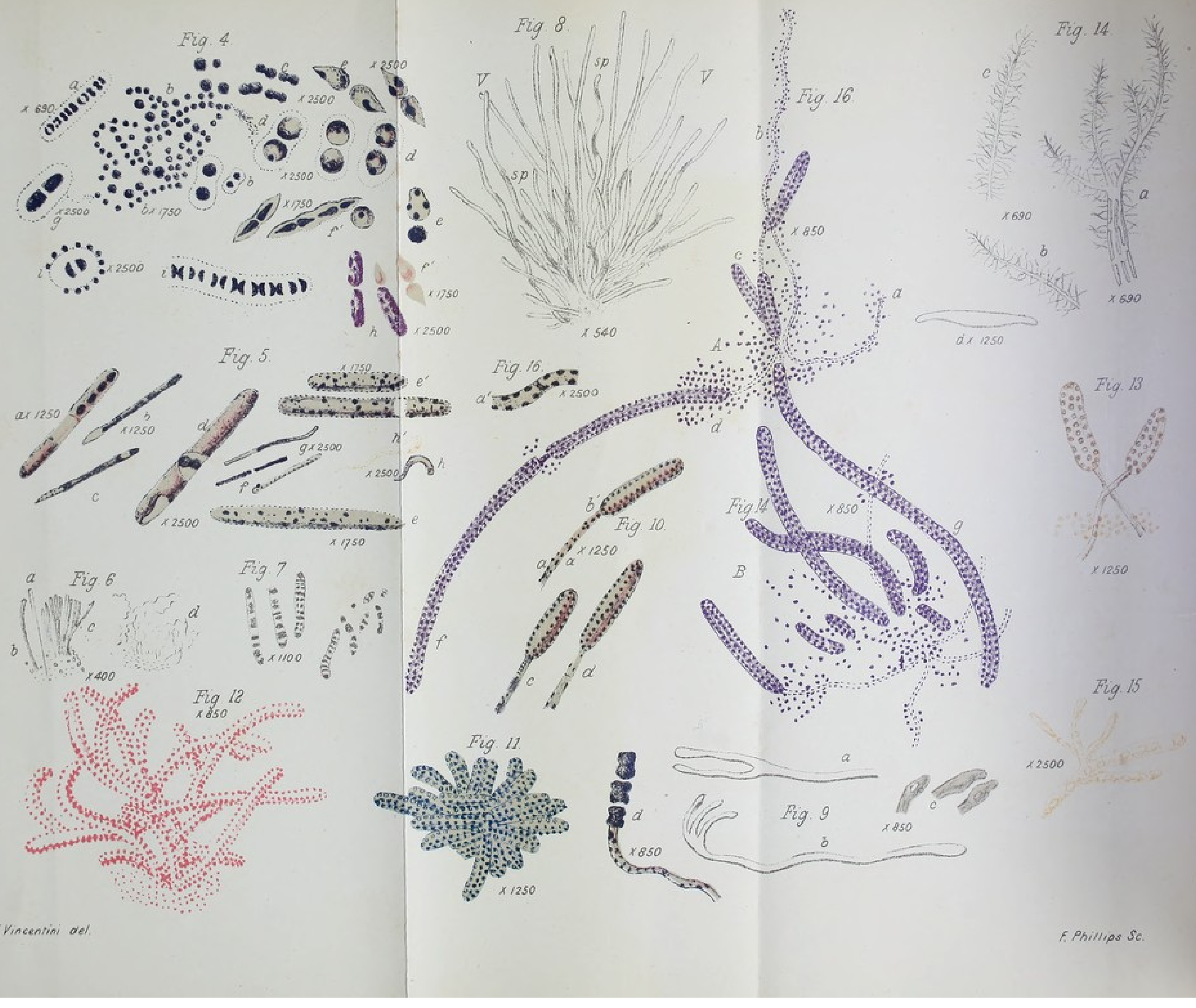
X.—That gentian violet is to be preferred in the examination of sputa in the fresh state, and this because of its double staining value; the myelin appearing blue, and bacteria and other morphological elements violet. The solution of iodine, picric acid, and fuchsine come next in point of usefulness; at the same time carmine has its special use in showing the internal formation of the vesicular gemmules of one of the fungi.

These conclusions are based on the examination of nineteen specimens of sputa, taken in fifteen cases of whooping-cough, out of which I have given particulars of nine. This is not a large number, but these epidemics of whooping-cough are not frequent, and I may have a long time to wait to renew my researches, and gather still further information. I have therefore preferred, without any delay, to bring what facts I have already learnt before this august academy (R. Acad. Med.-Chir., Napoli), that others, when the opportunity offers, may be urged to verify the results of my observations, and ascertain positively whether or no the abundance of myelin be a constant feature of whooping-cough, or be due to the peculiarity of a single epidemic, which by chance has fallen under my notice.*

To any thoughtful mind the question must arise, How could such an abundant flow of myelin, as I have described, fail to be noticed by preceding observers? How is it that it has never been mentioned by any of the many authors I have quoted? I have asked myself this question, and it seems to me that the only answer is that either past examinations have not been fully carried out, or that the presence of myelin has been treated as a fact of little importance, or else that casual phenomena has drawn me into error. Finally, I should recommend that anyone who desires to investigate into this matter should remember that myelin may be scarce, or even non-existent in sputa during the first or catarrhal period of the disease, and also during the close of the attack. And to bear in mind, too, that the scarcity or absence of myelin during the convulsive period is not an argument against my hypothesis, viz., the lesion of the medullary sheath of the nerve-fibres of the mucous membrane of the air passages. For this may be affected, and yet myelin be kept from flowing into the air passages whenever the minute bronchiæ are solely attacked by the disease, or when, by chance, a portion of sputum be examined coming from these smaller bronchiæ. A final opinion cannot be formed unless there is every certainty as regards the origin of the sputum, and no doubt left that it did proceed from the throat merely, or from the nose; in such cases it is but natural that no myelin would be found. There may even be cases where myelin is totally absent, and this not because the nerve-fibres are intact, but because the irritation has not reached the point of breaking up the medullary sheath from which the myelin is given off.

* The present memoir had already been prepared when, at the end of September and the beginning of October, there reappeared in this town several cases of whooping-cough. I have, therefore, been able to compare my observations, and find that they entirely confirm my previous conclusions, particularly with respect to the myelin and the fungi; should there at any time arise any facts which may induce me to modify these conclusions, or merit any especial consideration whatever, I shall be pleased to bring the same before the notice of the academy.

Plate 2.




F. Vincentini del.

F. Phillips Sc.

# Recent Bacteriological Researches on the Sputa; the Morphology and Biology of the Microbes of the Mouth.

By Filandro Vicentini, M.D., Chieti, taly.

*Translated by Professor E. Saieghi.*

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Further Remarks on the Bacteria and Bacilli found in the Sputa,

and their relation to the "*Leptothrix buccalis;*" the result of original observations on the morphology and biology of this parasite, principally on its higher phases, now for the first time examined and described.

*(Continuation of the Memoir on the Sputa of Whooping-Cough, Vol. XLIII., of the "Atti Accademia, Medico-Chirurgica di Napoli.")*

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"The manner of reproduction and the reproductive bodies of the *Leptothrix* are not known; but, perhaps, more accurate research will discover them: this hypothesis being based on the fact that, in the interior of filaments magnified to 800 diameters, small, round bodies are seen which might be spores."—Robin.

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Summary:

§ 1.—Further remarks on bacteria and bacilli found in the sputa and contents of the mouth—A *résumé* of the preceding work—Some forms of bacteria not therein described—Other forms of bacilli, *ut supra.*

§ 2.—Summary of the present investigations and methods of technique—Collecting and preparing the sputa and the buccal contents.

§ 3.—Previous opinions on the micro-organisms of the buccal cavity, according to Miller—History and general facts—*Leptothrix buccalis (Leptothrix innominata,* M.)—Other buccal micro-organisms—Buccal pathogenic fungi.

§ 4.—Observations on the morphology and biology of *Leptothrix buccalis*—The four phases of *Leptothrix*, particularly the fructification by spores—Production by *points* (male organs, ?) —Fructification of spores reproduced in the sputa—Dissemination of microbes in the posterior organs.

Recapitulation. Bibliography.

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§ I.

## FURTHER REMARKS ON THE BACTERIA AND BACILLI FOUND IN THE SPUTA AND IN THE CONTENTS OF THE MOUTH.

AT the meeting of 25th November, 1888, I had the honour of presenting to this illustrious Academy a memoir on the sputa of Pertussis, in which I incidentally touched upon sputa of another character.

It is not my intention to re-open all the questions before treated, but simply to refer to the bacteriological aspects. The recent observations refer to all bacteria and bacilli found in normal sputa as well as in those of various morbid conditions, the so-called tubercular bacilli not excluded, it being understood that the many special questions belonging to the latter bacilli will be fully considered later on.

Confining my remarks to the bacteriological researches in sputa, I will briefly repeat the conclusions I came to in the preceding memoir :—" The bacteria generally found in the sputa, and particularly in those of Pertussis, or Whooping-Cough, are derived, in all probability, from germs originating in the mouth and nose, so that (and until irrefutable proof to the contrary) their presence is to be taken as a natural fact, which has nothing to do with the cause of the disease. Such bacteria, although of very varied forms, can be reduced to two sole types, representing as many phases or forms of transition. One group may be identified with *Bacterium termo* and the other with *Leptothrix;* and probably even these two groups belong to a single species."

And elsewhere :—" In summing up the feature of the different types, we may conclude that the many forms of Bacteria delineated in our plate, together with the others simply pointed out in the text, can be reduced to two species, viz. : *Bacterium termo* and *Leptothrix.*

The forms *a*, *b*, *c*, above (Fig. 2, Pl. I.), *d*, *e*, *f*, *f'*, *g*, *i*, *k*, *l*, *l'*, *p*, and *p'*, apply to *Bacterium termo*; the forms *b'*, *c* (below), *m*, *n*, *n'*, *o* (left), *q*, *r*, *s*, *u*, *v*, *v'*, *x*, *x'*, *y*, and *z*, to *Leptothrix*. And it is not improbable that, in their turn, the two species, according to Cohn, Naegeli, De Bary, and others, may form only one. These authors classified the round *Bacterium* (*monas crepusculum*), *Bacterium termo* and *Leptothrix*, as three forms or developments of one and the same species.

I am rather inclined to believe that even the forms known under the names of *Vibrio rugula*, *clostrium*, and *rhabdomonas* may be attributed to the same species, as three simple varieties of its bacillary forms." I base these conclusions upon indirect signs, and especially upon certain morphological characters, common or transitional, on the usual habitat, etc.

Now, having followed up this line of research for the last two years, I think I may be able to give a direct demonstration of my work, by indicating, so to speak, the explanation of the polymorphism of the various microbes lodged in the sputa.

Moreover, in the preceding paper, several types of Bacteria and Bacilli, for brevity's sake, were overlooked, as well as other specimens drawn from further observations.*

* Whilst again touching on Whooping-cough, and in continuation of our bibliographical notices, we may mention a late article from Mircoli on "Renal Alterations in Pertussis" (*Arch. p. le Sc. Mediche*, 1890, p. 63, etc.) Mircoli inoculated, ineffectually, the cultures of bacteria, taken from the larynx of children who had died from Whooping-cough, into the larynx or under the skin of rabbits. He mentions articles from Sseurtschenho and Wendt, confirming the observations of Afanasieff. In the *Journal of Microscopy and Natural Science*, edited by Alfred Allen (January, 1890). there is a Lecture given by Dr. Shingleton Smith before the Microscopical Society of Bristol ("On Some Recent Developments of the doctrine of a Contagium Vivum"), where (at p. 32) it is said that the efficacy of special micro-organisms in most of the contagious diseases, and particularly in those most common to children, as *Measles*, *Whooping-cough*, and *Scarlet fever*, is not proved.

**Some forms of Bacteria not described in the preceding Memoir** (Fig. 4).

All forms of Bacteria and Bacilli herein described (Figs. 4 and 5) are coloured with gentian violet, excluding the filament or serpentine bacillus, *h'* (Fig. 5), which has been stained with iodine solution.

(*a*) In the preceding Memoir I maintained that the typical curved Diplococci—*g*, *l*, *p* (Fig. 2)—which are morphologically identical with the Gonococci of Neisser, never present a capsular appearance, and this proves to be so in most cases. But from further observations I have found exceptions to this rule, as is shown in Fig. 4*a* (magnified to 690 diameters). Here, in fact, the curved Diplococci are intermixed with straight Bacteria, on parallel cross lines, and are enclosed with the same envelope, which contains the whole. I found on December, 1890, similar specimens for the first time in the thick, opaque sputa of a child of seventeen months affected with Whooping-cough, and on the seventh day after the expectoration. The sputa contained, in the midst of common bacteria and bacilli, a fair number of red blood-corpuscles with a few ellipsoidal epithelia and granules of myelin. But, later on, I accidentally met with similar specimens in urine. This is no cause for surprise, as I believe that the impregnation of microbes in the urinary and spermatic passages to be the same as that of microbes vegetating in sputa, as they proceed from the same parasite, *Leptothrix*, which vegetates both in the mouth and in the external genito-urinary passages, as I shall prove later on.

In the third section I hope to show likewise the analogy between this type of Bacterium and the *Jodococcus vaginatus* of Miller.

But, besides these curved diplococci, which are intermixed with straight bacteria in a common envelope, others are to be found coupled only and enclosed in one sheath by pairs (seldom) in the *patina dentaria* (white deposit upon the teeth) around the rich colonies of Diplococci. Their capsular appearance is made striking, owing to the staining with picric acid and successive saturation in glycerine.

*b*, *b'*.—This specimen (magnified to 1750 diameters) is taken from the *patina dentaria*, mixed with saliva ; groups of diplococci are seen in it, both large and of medium size, interwoven with strings of very minute cocci, morphologically identical with those of type *s* (Fig. 2).

Such specimens, as well as diplococci with the capsular appearance of type *l'* (same figure), reproduced in *b'* (Fig. 4), are numberless. These diplococci are of two sizes, large or medium of type *l'* (Fig. 2) and others, surrounded likewise by a clear sheath, but small, as in *b'* (Fig. 4, right). The small cocci enclosed in *b'* do not differ materially from those of the beaded forms (with the exception of being surrounded by a sheath). I have observed that these minute diplococci are the heavier, because they are found mostly on the slide, whilst the large diplococci adhere to the cover-glass.

Now, the specimen drawn in *b* shows that the beaded forms, morphologically identical with the bacilli of Koch, are found in the *patina dentaria*, as well as in the solid and stagnant substrata, viz., within the buccal epithelia or in the morbid secretions, either in the crypts of the larynx or the tubercular products, in so far as the inactivity of the part favours the multiplication of the lineal series of cocci, which first form strings of beads and then small rods or filaments, as I have already demonstrated.

In the second place, from this specimen, I believe there exists a close affinity between those beads and diplococci, either of the buccal cavity or of some other habitat; otherwise I cannot understand why they should be so close together as to resemble fruits fallen from the same tree.

Another example of the tendency those minute cocci have to adhere to the diplococci is seen in *i*.

(*c*) The couples of dumb-bell bacteria herein shown (magnified to 2,500 diameters) are taken from the nasal mucus in its normal condition. I contend that they do not differ at all from the ordinary dumb-bell and chain-like bacteria found in the sputa upon the types *c*, *d*, *e* (Fig. 2), which remind us of the form and size of *Bacterium termo*.

On examining thick nasal mucus, I met with myriads of such bacteria. In this case the material to be examined must be taken from the dry cavities of the nose by a glass rod.

We shall see that those dumb-bell bacteria are more apt to form chains; in fact, they constitute the remarkable groups or bundles of chain-like bacteria found on the surface of the tongue.

According to my researches, each dumb-bell is the result of two cocci linked together by their heads. For this reason, it is often found, enclosed by a sheath, either inside or outside of the epithelia of the mouth, nose, or urethra. It is this adhesive tendency which forms them into chains or groups, from which in less moist parts will germinate the growth of *Leptothrix*, as we shall see later on.

I must refer to the preceding Memoir for the other forms of bacilli and bacteria, all related to *Leptothrix*. With regard to the nasal mucus, its pavement epithelia are smaller than the same epithelia of the mouth and less softened, perhaps from not being under the dissolving action of the saliva.

The bacteria which invade the pavement epithelia of the urethral mucus, taken directly from the meatus, often resemble dumb-bell forms. However, in the blenorrhœgic flow, are often found rather small diplococci, some in the pavement epithelia and others within pus corpuscles ; but curved diplococci or gonococci of Neisser are rarely found in it. Beside these common forms, I also detected very minute cocci, like those rosaries of type *s* (Fig. 2) or *b* (Fig. 4), and filaments or short articulations of *Leptothrix*.

*d*, *e*.—I have said before that the features of pneumococci of Friedländer, or Fränkel, etc., were probably derived from a degree of colouration of some points of their single articulations or from modifications of forms, as types *d* and *e* will show.

In *d* (Fig. 4, magnified to 2,500 diameters) are seen three sheathed diplococci, partially coloured.

In certain cases of pneumonia, we found some specimens of these, intermixed in the same group or colony.

In *e* the diplococci have no sheath and show points of varied partial colouring. Such specimens were obtained from a pulmonary sputa, in which, amongst other forms of bacilli and bacteria, were pneumococci of types *f*, *g*, *h*, *i*, *i'*.

*f*, *f'*, *g*.—These are specimens of the second kind, taken from the same sputum in a case of croupal pneumonia. In *f* (magnified to 1,750 diameters) the capsular appearances are so well surrounded as to resemble a capsular membrane. The single pneumococci are sometimes in groups of eight, ten, and fourteen. The internal articulations are prolonged and end in points.

In $f'$ (magnified to the same degree) the articulations are also acuminated, but have no halo, and at first are very pale. In successive days they become partially coloured in the preparation if kept artificially moist. These pale *Diplococci*, without sheath, appeared to be in a state of active germination, as we detected therein the more minute and proportionally paler forms, some being composed of unequal cocci, and several were moving about in the medium. The forms surrounded by sheath are firmer or less active, as if the capsule indicated the quiescent state of the microbe. In fact, if it moves, it is by a motion of simple translation, without vibrating, as is the case with bacteria in active germination.*

It appears that the capsule, owing to its great refraction, and also to the difficulty the near small bodies find in crossing it, resembles a fatty substance surrounding the microbe or proceeding from it. This is only an hypothesis.

In *g* (magnified to 2,500 diameters) is shown a distinct diplococcus, but with the internal articulations nearly cylindrical, with a large and somewhat irregular capsule, resembling the mono-articulated bacillus of type *q* (Fig. 2). The sheath indicates, even here, a quiescent state of the microbe and the successive secretion of pale substance. Some analogy may be found in it to the *Bacillus crassus sputigenus* of Kreibhom. (See section 3.)

*h*.—Intermixed with the diplococci *e* and $f'$ we find, at times, in the pulmonitic sputum, these forms without a sheath (magnified to 2,500 diameters). In one of these forms we infer the process of increase by seeing the pointed articulation at the right part as pale as in $f'$. The lengthened part of the mono-articulated bacillus is strongly coloured, and, in a good light, exhibits internal punctuations of deeper colour, especially near the edges : a remarkable circumstance that makes them resemble the *Leptothrix*, in which are also these punctuations or future gemmules.

The above forms are met with in the second period of pulmonary affections after the fever has subsided, but may also be

* In a recent work, Billet states he has met in other species with this form of incapsulated diplococci, and considers it a phase of life common to various species of bacteria. He calls it the zoogloeic state. (This will be again referred to in a note at the end of Section 3.)

found in successive periods. These pneumococci retain tenaciously the gentian violet, as I stated in the first memoir.

Several of the preceding forms of pneumococci, particularly those of types *d*, *e*, *f*, *f'*, and *h*, are analagous with those drawn by Cornil and Babes in *Les Bactéries et leur rôle*, Pl. XV., Figs. 1, 4, 6, and 13.*

*i*, *i'*.—The large round diplococcus, *i* (magnified to 2,500 diameters), was also found in the second period of a pulmonary affection. Its articulations resemble two detached hemispheres, and round the envelope adhere very minute cocci, not one of them being able to penetrate the clear zone. This fact clearly indicates the solidity of the sheath.

In *i'* there is a small *chain-like* bacterium, the articulations of which exhibit a series of similar diplococci, so connected that the hemisphere of the one adheres to that of the other, leaving a clear space between the hemispheres of each diplococcus. Such *chain-like* bacteria are sometimes found grouped together, although in small number, in pneumonic sputa.

### Other Forms of Bacilli, *ut supra* (Fig. 5).

Some forms of bacilli were omitted in the other memoir for want of space; others were collected later on, and these I will now briefly describe. The new forms added to the first will better show the identity of the bacilli of the sputa with the articulations or fragments of the filaments of *Leptothrix* vegetating in the mouth.

In the preceding Memoir the bacillus *n'* (Fig. 2) represented a form of transition from the young bacillary forms, *n*, *n*, *n*, to the mature forms, *m*, *m*. I then pointed out other forms of transition.

*a*, *b*, *c*.—In *a* (Fig. 5, magnified to 1,250 diameters) is seen a bacillus that partakes of three distinct types, divided into three segments: one internally occupied by four ellipsoidal bacteria, the clear segment in the middle, slightly coloured, and the other deeply coloured and opaque. It shows that the clear bacilli inside—those containing bacteria and those entirely opaque—belong to the same species. This bacillus was taken from influenza sputum, but similar forms were found in other sputa and in the saliva.

* Work quoted in the Bibliographical Appendix.

Another analogical proof we have in *b*, where a bacillus, opaque for five-sixths of its length, exhibits the other one-sixth clear and slightly coloured. This specimen was taken from the saliva.

In *c* we find four types—viz. : a short, clear segment above, and on the top of it a bacterium of elliptical form ; in the middle, a long, opaque segment ; and in the lower part a pale, granular segment like the middle feature of type *n'* (Fig. 2) and types *e* and *e'* (Fig. 5). This specimen was also taken from the saliva.

*d.*—This short but large bacillus, taken from the *patina dentaria* (magnified to 2,500 diameters), exhibits the upper part opaque and deeply coloured ; the other part is divided into two segments. The middle segment is very short, clear, and contains a dumb-bell bacterium placed across ; the lower one is opaque, but not entirely, as there is on the right a clear space between the opaque part and the outline, and presents a kind of indentation. Other bacilli have the same features towards the edges, or near the junctions of their segments.

As regards the enclosed bacterium, there is no doubt about its position in the envelope of the bacillus, from the look of the specimen as well as from the oscillation of the preparation, in which the bacterium was seen to be always inside. In the preceding specimens we have also abundant proof of this fact.

Now in the first place, it shows that not only elliptical, very minute, and linear bacteria, but also *dumb-bell* bacteria, may be found in the envelope of the bacilli ; from which we conclude there is no difference between the *dumb-bell* bacteria and the bacilli. And since the small chains, and the bundles especially, adhering to the tongue, are mostly formed of dumb-bell bacteria we must infer the analogy of those forms with the bacilli, and consequently of both to the *Leptothrix.*

In the second place, comparing the various specimens of bacilli (as *a* and *d*), containing bacteria with the fertile filaments of *Leptothrix*, we find in the latter a variable disposition of internal granules, or buds, adhesive to the envelope. Some are alternate ; others on the same level at both sides. If we imagine that in those filaments the buds grow up towards the middle line of the stem, it will result that, when the left bud is placed higher than that on the right, the bacteria resulting from their increase will range themselves in a longitudinal line, taking the elliptical form, as in bacillus *a*. On the contrary, if the two buds are on the same level, we shall have the case of bacillus *d*—viz., the two cocci, resulting from the increase of the buds, will meet in the centre of the stem, and, there joining together, will produce, in a cross line, the *dumb-bell* bacterium.

Therefore, wherever we turn our eyes, the notion of the polymorphism of the microbes, and their probable derivation from a single species, is gaining firmer ground.

*e*, *e'*.—The bacillus drawn in *e* (Fig. 5, magnified to 1,750 diam.) was found in the sputa of a young woman in labour, who was affected with bronchitis. It was mixed with other similar granular bacilli, clear and of the same length, but more slender, as we see in *f*. These bacilli, however, are common also in other sputa and in the contents of the mouth; they present a pale, granulated mass, similar to that of bacilli, *n'* (Fig. 2) and *c* (Fig. 5). They also show, in various places, buds or internal granules, smaller and paler than those found in the fertile filaments of *Leptothrix*.

In *e* the ends are pointed, but in *e'* they are round. The larger of the two is faintly coloured, but exhibits near one of the heads a clear interstice, as is seen in *a* and *d*. The other specimen, smaller and paler, exhibits a younger form of the same type. These two last bacilli were found in pulmonitic sputa, and were also found in the contents of the mouth.

In the uncoloured preparations of the *patina dentaria* there are *nail-shaped* bacilli, like the type *e*, soft, flexible, slightly veined, and having quick motion (Fig. 14, *d*). These bacilli are originally lodged on some pointed productions, as was observed by me for the first time; their slender points form the bacilli *f*, *h*, and *h'*.

*f*, *g*, *h*, *h'*.—The very slender bacilli, *f* and *g* (magnified to 2,500 diameters) are also taken from a case of pneumonia. In *f* we see a mono-articulated and strongly coloured bacillus, as well as another pale and granulated. In *g*, a very slender one, the ends of which appear to have been recently detached from a longer filament.

By comparing these specimens with the *Leptothrix*, no important difference is noticed.

In *h* another specimen of curved bacillus, resembling a point of interrogation, taken from pulmonitic sputum. These bacilli are not uncommon in the *patina dentaria*. They have quick vital movements. They resemble the *Spirillum sputigenum* of Miller or the *Comma bacillus* of the saliva described by Lewis, as identical with the cholera bacillus of Koch, *Bacillus virgola*.*

I have, however, found that, between these bacilli and the lesser points, which are abundant on certain filaments of *Leptothrix*, there exists a great similarity. These *points* vary in size, but all possess quick movements (Fig. 14). We shall consider later on if these bacilli act like the reproductory filaments (spermatia or antherozoids) of many cryptogams, although in *Leptothrix* they are not originally contained in special receptacles (spermogones or antherids).

In a more advanced hypothesis, we shall have also to consider if these bacilli represent the spirilli, or if the spirilli proceed from such filaments, endowed with the same motile power. It appears more probable that the spirilli may generally be derived from the fragments of certain curved filaments, fertile or not, of *Leptothrix*. Sometimes the spirillum is thicker than the filaments of *Leptothrix*, as in Fig. 1 of Cornil and Babes.†

In *h'* is seen a larger filament (stained with iodine), found in the *patina dentaria*, which is somewhat similar to spermatozoa.

In the uncoloured preparations I have seen the very rapid movements of all these bacilli. In conclusion, all these forms of bacilli are common in the expectorations and contents of the mouth.

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## § II.
## SUMMARY OF THE PRESENT INVESTIGATIONS AND METHODS OF WORKING.

ARGUMENT.

In the fourth section of my Memoir on Whooping-cough, I stated that bacteriological researches on the sputa would be fruit-

* Lewis, *A Memorandum on the Comma-shaped Bacillus, etc.*

† Cornil and Babes, *op. cit.*—Vicentini, "Sopra un caso di febbre ricorrente ecc. e sul riscontro degli *Spirilli* nel Sangue," *Atti del. R. Accad. Med. Chir. di Napoli*, 1883, t. XXXVI.

less until the natural history of the pathogenic or non-pathogenic microbes that lodge there is thoroughly known.

In most cases a complete organism grows in length, breadth, and thickness. But the chain-like bacteria and the filaments only grow in one direction (in length) simply by a repetition of particles in a lineal series. We must, therefore, suppose that neither these original particles, nor the *chain-like* bacteria and filaments resulting from them, are complete organisms, but that they are rather rudiments of more perfect ones.

By examining all the different forms of bacteria and bacilli, we were led to believe in a single type, to which all other forms were related. Now, we will show that the existence of this vegetation is to be found in the *patina dentaria*, in certain sputa, and in the mucus of the urethra, all the forms hitherto described being only fragments of it.

If somebody, for instance, not knowing all the phanerogams, should enter a wood-house, looking at the wood, heaps of branches, leaves, etc., he might at first suppose that they were different things instead of parts of a whole. Likewise, if he should see a threshing-floor upon which is spread and beaten Indian corn, he might take the stems, roots, leaves, tufts, and grains for as many different objects. But it will be sufficient to take him out into the field, to convince him of his mistake. Looking through the lines of the corn, he would see that most of the plants (the female ones) are provided with ears, and he would see also others higher than the rest (the male ones), having instead of ears only a tuft.

Then he might suppose that the plants of this second type were, perhaps, of different kind from the others, not thinking that Indian corn is a diœcious plant, with distinct sexes on different individuals.

This is precisely the case with the microbes of sputa. We were led to the study of the morphology and biology of *Leptothrix*, by considering that nothing is useless in nature ; those parts which now appear superfluous to us in living organisms, (as the nipples in man) are rare exceptions.

The illustrations of *Leptothrix* contradict this law. Look, for instance, in Bizzozero, the figure of *Leptothrix buccalis*, reproduced in our Fig. 6, and the query will be asked :—Of what use are those upright, barren shoots resembling grass, which have been eaten close by cattle? Cannot this want of fruits be accounted for by mechanical injuries? The figures of Robin, Frey, Miller, and others do not suggest a different conclusion.*

After these reflections, we were induced to investigate this parasite. Our researches will be particularly demonstrated in Section 4, only a synthesis of them being given here.

From our observations *Leptothrix buccalis* would, therefore, live under four forms, or have four different stages of development, according to the conditions of the alimentary substratum.

In the liquid secretions, as saliva and mucus, its spores—bacteria and cocci—and its gemmiferous sprouts—real bacilli (excluding the Spirilla, the *nail-like*, the *snake-like*, and the *comma*)—increase and multiply like moving spores and germs of fungi. This would be *the immersed vegetation of Leptothrix.*

The bacteria, the yeasts, and the spores have generally the power of reproducing the full organism like the seeds of the phanerogams; but, between the spores of the bacteria on one side, and the seeds of the phanerogams on the other, there is this difference: that those seeds cannot multiply by division, producing fresh seeds; whilst moving spores, yeasts, and bacteria multiply abundantly, by fission, even by themselves.

If, however, the liquid is kept quiescent in the crypts or cavities, or if the part is, as on the surface of the tongue, very firm, the second period or low vegetation begins, viz., *beaded*, *chain-like*, and *bundle-like* bacteria are formed, so common on the patina of the tongue. But vegetation stops here, for in the mucous parts the continual friction impedes a further formation.

In the secretions and mucus there is a relative inactivity; but if it becomes effective, a reproductive growth will be formed, as shown in Fig. 16, taken from pulmonitic sputum, which for several days had adhered in the interior of a closed tube.

The third period, or growth, is when it lodges on a solid part,

* Bizzozero, *Manuale cit. nella Bibliografia*, Pl. II., Fig. 27; Robin, *Histoire naturelle des végétaux*, etc., 1853 (see Bibliography), Pl. I., Figs. 1 and 2. Frey, figure reproduced by Perroncito, quoted in Bibl., Fig. 11. Miller, figures quoted later on.

on the tongue or teeth. But, owing to the continual friction on the tongue and teeth, the growth cannot reach the fourth period, or that of fructification. Then we have bare growths apparently sterile, as represented above. The same happens with the tufts of *Leptothrix*, often found on the epithelium scales, on the residue of undigested food, or other corpuscles in urine, sputa, etc., forms which may be called "aërial," although incomplete, "vegetation."

But in many parts of the dental surface, where there is less friction, as between tooth and tooth and near the gums, the fourth phase or fructification takes place. This fact has not been observed, and is not even suspected, as we gather from the most recent work of Miller.*

I call it aërial vegetation, because the stems of the microphite have a tendency to rise in the air, although immersed like the algæ. In our case the surrounding liquid is the saliva. This fructification by spores, first noticed and investigated by me, consists of comparatively long ears (Figs. 10, 11, 12, 13, 16), formed by the exudation of a viscous substance, faintly coloured, round the ends of fertile filaments. In this substance are entangled small round spores, often brightly coloured, placed in six longitudinal lines, as we shall see later. In the strongest specimens (Fig. 16, *f*, *g*) as many as 720 spores can be counted in an ear. All the cocci and minute bacteria are probably disseminations from these spores.

There is another form of special production, or pseudo-inflorescence, which is drawn in Fig. 14, *a*, *b*, *c*, resembling *points*, round a filament or group of fertile filaments. These *points* having fallen off form the *nail-like*, *snake-like*, or *comma* bacilli. If we admit the analogy of these bacilli with the reproductive filaments of other cryptogams, *Leptothrix* may be compared to a fungus, or *diœcious alga*, with two sexes upon different filaments; and such pointed productions would exhibit the male organs, the elements of which, spread about the surrounding medium, would, with their great mobility, act as antherozoids in a manner not yet known to us. In such an hypothesis, the scanty number of such inflorescences, with regard to fructification or female organs, would be fully explained. Therefore,

* W. D. Miller, *Die Mikro-Organismen der Mundhöhle*, etc., 1889.

in summing up the morphological and biological series of *Leptothrix*, beginning with the forms of Cocci or Bacteria, we pass through the chains, the growths, and end in the fructification by spores, in order to begin again another series *usque ad infinitum*. But, beside this cycle of reproduction, we must notice another—of buds and internal impregnation (that already foreseen by Robin), which is seen in the interior of the envelope of old filaments, articulations, and bacilli, and through which, from the same bacilli, are generated other bacteria, and this owing to the reserve buds described above. From those bacteria are, in their turn, reproduced the chains, the tendrils, and their articulations or bacilli, as we have seen in the former Memoir on the subject of bacilli *b'* and *n* (below) and in *u*, *u* (Fig. 2).

Let us consider what a great cause of dissemination this is, placed at the entrance of the digestive and respiratory organs. According to our calculations, there would always be found present in the nose and mouth, taken together, from two hundred to three hundred trillions of bacteria, or other elements of the microphite, ready to enter the stomach at the time of deglutition, and to fall on to the respiratory passages at every breath. In comparison with that mass, what are the few germs we inhale from the atmosphere?

But if, at every breath we inhale, a propelling motion sends whole swarms of elements or germs of the microphite into the air-passages, another slower but continuous movement pushes germs or analogous elements on into the genito-urinary passages through contiguity, from one epithelium to another, and onwards along the mucous patina, passing through the urethral orifice, which Pasteur has compared, in this case, to the Thames Tunnel.*

We will briefly treat these questions in Section 4, and demonstrate the identity of *Leptothrix preputialis* with *Leptothrix buccalis*. Now, to enable others who may be willing to repeat and to verify our observations, we will proceed to describe the *modus operandi*.

* Schutzenberger Le fermentazioni, ecc. trad., Milano, 1876, p. 204; V. also Vicentini Caso di vegetazione di funghi microscopici nell' Uretra ecc, Morgagni, April, 1880.

## Collecting and the Preparation of the Sputa.

In the preceding work we dealt with the collecting and the treatment of sputa, putting off to another occasion the diagnosis of derivation of the various materials expectorated, in order that the real sputa might be distinguished from the particles of food; the sputa from the saliva or throat, and the mucus flowing from the nose, being also differentiated. Now is the moment to touch on this point again, in order to describe improved methods of preparation and colouring.

The materials to be submitted to microscopic observation really do not always proceed from the air-passages. In the case of a boy, 7 years old, affected with Whooping-cough, we remember having received a portion of food ejected from the stomach, and thought to be a sputum. This substance was gelatinous in appearance, the colour of tobacco, and contained a large amount of alimentary residue; but what surprised us was a very dense mycelium of conspicuous sprouts after the type drawn in former plate, Fig. 3, *d*, interwoven with other slender ones. The fructification of these mycelia produced forms identical with small heads (capitula) or sporangia, delineated in *k* and *k'*, very vigorous, upon as many as five fertile branches, intermixed with similar vigorous forms of *Aspergillus glaucus*. This would lead us to suppose that the deglutition of the relative germs with the sputa, and their secondary development, is even more easy and vigorous in the stomach.

In some cases, especially with children, the proper expectoration is lost or swallowed; and when the patient is asked to spit out again, he emits only a little saliva, mixed with small flakes of mucus, from the throat and tonsils. These salivary sputa, however, may be recognised generally on the following day through the clarification, after remaining undisturbed, with an upper liquid stratum (more considerable than in crude sputa, or those mixed with abundant saliva), and a scanty deposit, very rich in buccal epithelia, and, eventually, alimentary residue. Besides, they are recognised from being wanting in ellipsoidal epithelia and granules of myelin.

Another source of error, against which it is difficult to guard, is the reflux of nasal mucus, through the posterior nares, in the back of the mouth, and its successive expectoration mixed with saliva. Naturally, ellipsoidal epithelia and myelin, even in such sputa, are wanting.

In any case the presence of the myelin and the ellipsoidal epithelia induces us to regard the sputum as proceeding from the air-passages. These are two important points when, for instance, a doubt arises whether a sputum with a rusty appearance proceeds from the nose or the chest; as, in our daily researches on sputa, we never detected ellipsoidal epithelia or myelin in spurious sputa; whilst in the genuine expectorations they are very seldom absent, and when not found in the first, they appear in the second preparations.

We come now to the manner of collecting the sputa.

The act of spitting is accomplished in two different efforts: by the first, the genuine sputum, nearly entirely free from saliva, and that which is required is coughed up; but soon after by a second effort, saliva is emitted with the residue of the sputum left behind. This second sputum (being nearly all saliva) is eliminated. In order to avoid mixing the sputum with saliva, the patient should not keep it in the mouth too long.

Instead of a spittoon, it is better to use a colourless plate or saucer, perfectly clean, where the sputa can be kept separate, and then selected for examination. The sputum should be taken at the most viscid point with a bent needle or forceps, and raised so that the saliva and the more fluid part of the mucus with which it is mixed, may trickle down on the plate. What remains on the instrument is quite sufficient for successive investigations, and it will necessarily contain very little saliva. To preserve it, it should at once be placed in a glass tube, which has previously been cleaned with sulphuric acid, and washed out with alcohol.

For the coloured preparations we have to modify the directions given in the preceding work. It is better to keep the solution of gentian violet separated from the aniline water, so as to prevent decomposition; but we must avoid putting the glass rod, still wet with aniline water, into the colouring solution, and *vice versa.* Two different rods must be used. With a small rod put first on the slide a drop of aniline water, taken from the relative bottl , and soon after add a colouring solution. For, by placing the solution first and the aniline water afterwards, we obtain a greater precipitation of coloured granules in the preparation.

It is necessary that the particle of sputum should be well immersed in the colouring mixture, so as to have it well coloured on its under as well as its upper surface; because, if the particle is placed on the slide before the solution, the part adhering to the glass cannot be coloured. The particle of the sputum should not be larger than 1/4th to 1/3rd of a grain of millet; otherwise, when pressed between the two slips, it will press out from under the cover-glass, and consequently the best specimens, which are on the edges, will be lost, or the preparation will prove too thick for investigating the most delicate parts.

The particle of sputum, taken with a bent needle, should be immediately put into the colouring mixture, pressed down for a short time with the needle, then left for two hours under a watch-glass; a drop of distilled water may be added to it, if a weaker colouring be desired; or, in hot weather, to prevent hardening or evaporation.

After the required time has passed, take the sputum with a perfectly clean needle, put it in a watch-glass containing distilled water, and, by gentle agitation, it will be freed from the granular deposits. Meanwhile the slide must be well cleansed. Then put upon it a drop of distilled water, in which the particle is to be again immersed. By using glycerine we cannot obtain the proper thinness of the preparation, and many features would appear to be altered and colourless. Glycerine can ultimately be substituted by capillarity to preserve the preparation, the upper surface of the cover-glass, made greasy by the immersion, being washed first with benzine and then with water.

In the preceding Memoir, we recommended the spreading and thinning of the particle of the sputum with needles; but for our present investigation it would not be advisable to do so. It was, perhaps, owing to this that we then lost some details, especially with regard to the fructifications of *Leptothrix*. In fact, spreading with needles must destroy the relations of continuity and contiguity necessary to preserve intact the said fructifications.

To thin the preparation as much as possible, we must place upon it the cover-glass, well centred, and let it act by its own pressure. In this way the mucus becomes thinned, and spreads slowly without altering materially the elements involved in it. When the preparation has become partially thinned, press down lightly and gently with the rod and leave it undisturbed, and so on until the very slender film of the coloured matter has in every direction neared the edges of the cover-glass; care being taken in these handlings to strain off the redundant liquid without wetting the upper surface of the cover-glass.

The preparations so mounted, in water, are in all parts very transparent, clear, and bright; they do not exhibit superposition of elements or corpuscles, and even in hot weather, keep well under the microscope for several days, if care be taken to wet now and then one of the edges, as we suggested in the first Memoir. Even the bacteria in the centre of the particle of sputum which were not at first reached by the colouring medium become in time gradually coloured, and these weak colourings are often very useful for examining minute details.

In order to obtain fructifications of *Leptothrix* from sputa, it is necessary to take the particle of sputum, not from the bottom, but from that side of the tube where, perhaps accidentally, a vertical streak of the mucus, slender and adhesive, has been left, as we shall see later.

For researches on fructifications, powerful immersion objectives are required, so as to be able to detect all details. We have used a No. 11 objective (1/18th homogeneous immersion) of Hartnack.

### Collection and Preparation of the Contents of the Mouth.

We shall especially deal with the *patina dentaria* (surface of the enamel, tartar), saliva and patina of the tongue.

**Patina Dentaria.**—The deep layers of this patina contain almost exclusively bud-growths and knots of large filaments which take a brilliant colour with the solution of iodine. The fructifications, on the contrary, are simply found on the superficial layers, so that for their investigation we must possibly remove the upper surface of the patina. By scraping the tooth with a small instrument or the nail, too much tartar is removed, which must be disintegrated twice over; first to select a particle, and then to thin and spread it on the slide. I have best succeeded with a bent needle, first scaling lightly the labial surface of the tooth, and carrying the very minute particle of the patina on to the slide (on which was placed a drop of aniline water), and then scraping likewise the interstice between the teeth, to place on the slide another similar particle. In order not to spoil the fructifications, the scraping should be made neatly from top to bottom. This manipulation should be done in the morning, fasting, and cannot give good results in those subjects who habitually clean their teeth with tooth-brush and powders.

Thus we get within the drop of aniline water two tiny islands of *Leptothrix*, but they are yet too thick to be reduced to the thinness required. We shall break them with the needles, so as to carry on to the slide only a kind of whitish dust, which will occupy a third of the area to be covered. Having done this, we shall add with the rod another drop of aniline water, and soon after, with a different rod, a drop of gentian violet solution. We shall speak of the other colourings in Section 4, the treatment being nearly the same. The preparation is kept for a little while under a glass, and when it appears to the naked eye to be sufficiently coloured, it is mounted without washing, which, in this case, would take away all the fragments, the fructifications set free, and the bacilli floating about each tiny island.

When we wish to examine together the saliva and the *patina dentaria*, instead of placing a drop of aniline water previously on the slide, we will put on it a drop of saliva in the manner we shall indicate by-and-by; the rest of the treatment is the same. However, the preparations without the saliva turn out clearer and thinner; whilst those mixed with it cannot become thin enough, owing to the resistance presented by cumuli of large buccal epithelia, which place themselves like supports between the slides; still worse when one of those cumuli happens to be on a bed of *Leptothrix*. Naturally, in the preparations mixed with saliva, the rich cumuli of *Diplococci* are more abundant, whilst they are seldom found on those of the patina only.

Lastly, the cover-glass is put on and gently pressed down with the rod, until the layer of the *Leptothrix*, spreading in every direction, reaches the edges of the cover, and the redundant liquid trickles down by its side. The preparations, stained with aniline colours, become a little hard (perhaps owing to the alcohol in it); but yet they get thinned enough. Those treated with the iodine, and especially those treated with picric acid, or the colourless ones, being more flexible, become more easily thinned.

In order to see the fructifications in a fair quantity and distinctly, it is necessary to wait some time. Naturally, those on the edges of each tiny island are seen more distinctly, but all do not show them, because many of the islands contain only filaments or bud-growths. Some are wasted, and others overlie one another, mixing their relative edges together. Now, in those edges with exuberant fructifications, these at first are close together; but the preparation in time partially dries up, and on pouring on it a fresh drop of distilled water the cover-glass slightly rises, and then the tufts of ears begin to open; the ears separate one from another, and (in preparations with picric acid) several may be counted in the visual field. Such observations can be continued for several days by adding to the preparation from time to time a drop of distilled water.

To demonstrate the productions by points, the method of working must be modified, as we shall see in Section 4.

**Saliva.**—To get the saliva as far as possible free from froth and air-bubbles, the following plan should be adopted:—Let the patient before tasting food spit on a well cleaned glass rod, held horizontally; then incline it so that the saliva shall run down towards the hand, parting, in its course, from the small air-bubbles, which, being lighter, adhere to the rod and stop the froth. When a drop of saliva has gathered on the handle, it may be taken off by the point of a second rod and carried directly on to the slide (if we wish to examine it with the *patina dentaria* and then mix them together); or, if we wish to examine them separately, it should be immersed in a drop of colouring mixture, already placed on the slide. As soon as the preparation is sufficiently coloured, it should be covered without washing. If the colouring is very weak, with slight vestiges of colour, the vibrating motion in the salivary corpuscles will be better detected.

**The Cuticle of the Tongue.**—On an empty stomach, the surface of the tongue is scraped with a spatula or tongue-scraper. We take a small particle of the product and put it in the aniline water previously deposited on the slide. Afterwards it is carefully divided and coloured like the *patina dentaria*. However, owing to the superposed epithelia, these preparations can never be properly thinned, and therefore are only in a few points rendered clear enough for investigation under high powers.

We need not deal separately with the nasal mucus and the *smegma-balano-præputialis*, as the first is treated like the sputa, and the second like the *patina dentaria*. We shall only notice that the preparations of the smegma do not become satisfactorily coloured with aniline dye, on account of the abundant precipitation, due to fats; it is better to make use of iodine or picric acid.

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§ III.

## OPINIONS HITHERTO HELD RESPECTING THE MICRO-ORGANISMS OF THE MOUTH, according to Miller.

### History and General Facts.

WHEN I for the first time began to examine the fructification of *Leptothrix*, of which no mention had hitherto been made in the ordinary text-books of microscopy, I, of course, consulted several of the special publications quoted in the Bibliographical Appendix, in order to ascertain whether others had described these fructifications before; but my search was fruitless. It is true that Robin believed in their existence, as appears from the quotation at the beginning of this Memoir; but the granules mentioned by him are simply the reserve gemmules of this microphite. However, I should not have been induced to treat on this subject so early, had I not unexpectedly met with a very conspicuous specimen of such fructification in some pulmonitic sputum (see Fig. 16). I then wished to acquaint myself with the last work of Miller, already quoted, and on perusing it, I found that he had not even touched on that point; but, on the contrary, his views were, in a sense, diametrically opposed to my own.

Of the specimen found in the pulmonitic sputum I shall speak in Section 4. According to Miller, the opinions hitherto acquired on the microbes of the mouth would be as follows:—

In the 3rd chapter of his volume, "The Micro-Organisms of the Buccal Cavity: Local and General Complaints produced by them" (the most complete and most recent work on the subject), the illustrious Berlin Professor goes on to relate the ancient and recent scientific opinions on the microbes of the mouth, beginning with their discoverer, Leeuwenhoek, who, in 1683, discovered first on his own teeth, always kept clean, and afterwards on those of an old man, "*magna cum admiratione*," animalcules, drawn by him (Fig. 10 of Miller), some of which resemble the Comma bacilli:—"*Multa exigua admodum animalcula jucundissimo modo se moventia.*"

Lebeaume compared the tartar on the teeth to coral formations. Mandl held that the tartar proceeded from the chalky remains of the vibriones described by him, which he thought would be killed by heat, by hydrochloric acid, and alcohol. Bühlmann was the first to observe and describe the filaments of *Leptothrix*, without, however, giving any opinion as regards their vegetable or animal nature.

Henle was the first to declare that these microbes were of a vegetable nature.

Erdl treated the decayed teeth with hydrochloric acid, and from the crown he obtained a kind of delicate, enveloping membrane, composed of parasites.

Ficinus dealt diffusely with microbes of the buccal cavity, which he held to be of an animal nature, and gave them the generic name of *dental animalcules.* He described the filaments of Leeuwenhoek and of Bühlmann, the granules, the epithelia, the corpuscles of mucus, forming the *patina dentaria*, as well as certain infusoria that accidentally dwelt there. He not only held the bacteria and bacilli to be real animalcules in brisk motion, but he imagined also that they possessed a mouth. He grouped them with infusoria without cilia, probably shelled, after the types of the genera *Paramæcium* and *Colpoda.*

In 1847 Robin thought that the principal micro-organism of the mouth was an alga which he called *Leptothrix buccalis*.

Klencke, continuing the researches of Bühlmann and Ficinus, drew the dental animalcules, the filaments of Bühlmann (reproduced in Fig. 11 of M.); and, following the opinions of Ficinus, he even drew the mouths on their ventral side, as can be seen in the quoted figure.

Meanwhile, the ideas of Robin were accepted, and *Leptothrix* was generally held to be an alga or fungus *sui generis*, excepting by Frey, who, together with Hallier, thought it to be a form of *Penicillium glaucum*, and by Tilbury-Fox, who restricted it indiscriminately to a form of *Oidium*.*

After these historical hints, Miller goes on to describe his methods of investigations, from which it appears that the author and the preceding investigators have not acted with that necessary care and discernment, which has been demonstrated, in collecting and preparing the contents of the mouth; nor have they instituted comparisons with the sputa. They directed their best attention to the culture of the microbes of the mouth, and, in the study of the *patina dentaria*, they did not avail themselves of the immersion methods except in the special examination of certain fragments or isolated microbes and of their cultures. It is no wonder, therefore, that they should not have met with the fructifications and the productions of *Leptothrix* by points.

Miller speaks of cultures on agar or on calf's blood serum, and from thousands of specimens he gives the preference to agar peptonised with broth, with an additional 0·5—1 per 100 of sugar.

The cultures were made from saliva, the tartar of decayed teeth, or scrapings from the surface of the tongue or from dental ulcerations and altered pulps of teeth. The cultures from saliva in the gelatine were negative. Often the bacteria reared in agar did not succeed in gelatine, owing to the low temperature maintained.

For a general study, he considers an amplification of 20 to 300 diameters sufficient, but for the special morphology he used a homogeneous immersion lens. He speaks of fungi derived from

* Frey, Bibliography. Tilbury-Fox, by Beale, Work, p. 491.

external sources, as the Bacilli of tare, potatoes, lactic acid, green pus; and also of the *Micrococcus tetragenus*, of *Mycoderma aceti*, of *Staphylococcus pyogenes*, *aureus*, and *albus*, to the effect of excluding them from the cultivations. But he touches again upon some of these forms in Chapter IX., as we shall see later on.

Miller classifies, afterwards, the microbes really constant, or primary, of the buccal cavity, into six different species. Of these we give a list, putting our denominations to each species of Miller.

**Synopsis.**

| Miller: | Vicentini: |
|---|---|
| 1—*Leptothrix buccalis (L. innominata).* | *Leptothrix buccalis.* |
| 2—*Bacillus buccalis maximus.* | Fragments of stumps. |
| 3—*Leptothrix buccalis maxima.* | Stumps. |
| 4—*Jodococcus vaginatus.* | Special sheaths of bacteria proceeding from some reserve gemmules. |
| 5—*Spirillum sputigenum.* | Pointed or virgulated Bacilli (copulatory organs?) |
| 6—*Spirochæte dentium.* | Spirillum (fragments of very slender filaments). |

The above species or types are found in every mouth. The *Spirillum sputigenum* is sometimes found, in almost a pure culture, in the carious cavities; and the *Spirochæte dentium* near the gums. The cultures from substances mixed with saliva, acids, alkalies, product of caries, dental mucus gathered out of the ebullition of decayed teeth, do not succeed; some simple cultures, however, give productions of 15 or 20 articulations of certain species, but these cannot be reproduced a second time.

I will now sum up the characteristics which Miller attributes to these six primary species, beginning with *Leptothrix innominata.*

### Leptothrix buccalis (*Leptothrix innominata*).

The name of *Leptothrix buccalis* is attributed to Robin, who applied it in general to all buccal microbes. Hallier, Zopf, and all others who wrote on this subject conformed to his opinion, maintaining that the motile bacteria are spores of *Leptothrix* in activity, and cocci and bacteria not motile are wandering spores in a quiescent state.

Leber and Rottenstein found the fine violet staining with iodine and the acids to be a characteristic of buccal microbes (included by them in a single species of *Leptothrix*). Miller, however, considers such forms to be different species, although they show the same behaviour towards staining re-agents, for the reason that the filaments of *Leptothrix* are not articulated (as stated by Robin). On the contrary, the bacilli or filaments under iodine re-agents are articulated. Now, can this distinctive character be sufficient to classify a species? I shall show later that, besides articulations, bacilli may be found, or knotty little rods in continuity with small chains, and the latter with fertile filaments (not articulated), as in Figs. 9 and 10. This demonstrates the fact that such filaments or bacilli, either articulated or not, or simply in beaded forms or chains, do not constitute a difference of species. Moreover, the filaments more woody and articulated, found in the lower layer of the patina, or, owing to the friction, are left exposed, so that the remaining stems become thick and hard, and their internal gemmules give rise to enclosed bacteria. Vignal was not of a different opinion in holding a fungus of the mouth, which he had reared, to be *Leptothrix*, although it showed transverse segmentations easily discernible in aniline. Likewise, Miller, in his article on *Leptothrix gigantea*, as we shall see later, entertained the same opinion.

Quite recently, the dumb-bell bacteria and other fungi of the mouth were identified with *Bacterium termo* by Stockwell, Clark, and others. In the preceding Memoir we held this identity as plausible; but Miller positively rejects it because the title of *Bacterium termo* would embrace a mass of very different forms and species. He likewise rejects the title of *Leptothrix buccalis* (given by Robin) and of dental filaments (by Bühlmann), names which Miller would have banished from microphitology. However, he would give the name of *Leptothrix innominata* to those little-known filaments which appear to constitute a group or a species differing from other fungi. It is clear that the author alludes to our fertile filaments, making of them a separate species.

The *Leptothrix innominata* of Miller (the real *Leptothrix* akin to fructifications to our knowledge) is to be found in the *patina dentaria* (*materia alba* of Leeuwenhoek) constantly, but in different quantities in every mouth. It is, of course, very scarce in well-cleaned teeth, because, lying on the top of the tufts, it is the first to be removed by friction. The *patina dentaria* exhibits large and small heaps of round granules, and, on the edges, slender filaments variously bent. The granules were considered as the matrix of the fungus, having been taken for spores of *Leptothrix*. The author thinks them to be either micrococci foreign to *Leptothrix*, or simply linking points of its filaments.

The filaments are of varied length, from 0·5 to 0·8 micro-millimetres, sometimes twisted, usually still, and without articulations (but this is not always exact). They generally have irregular contours and appear to be badly nourished or are dead, but we shall see that they contain gemmules and bear spores (ears ?). In their surroundings there are also numerous shorter filaments or small rods, which the author thinks might be simply fragments of longer filaments, or cellules of the fungus not having yet reached their full development.

By using the solution of iodine, slightly acidulated with lactic acid, under a power of 350 diameters, epithelia and clusters of micrococci can be detected intermixed with them (Fig. 12 of Miller), and even various forms of little rods and filaments of *Leptothrix* lightly tinged with yellow. Other larger bacilli become tinged with deep violet, and these are called by the author *Jodococcus vaginatus* and *Bacillus buccalis maximus*.

The same author again speaks of the *Leptothrix innominata* on page 203. He declares positively that it cannot be cultivated in every medium. This is true, if he intends to speak of the ordinary culture media, but not equally so with regard to sputa, in which are to be found not only natural cultures, but even the most vigorous fructifications of this parasite, as we shall see later on.

### Other Primary Micro-Organisms of the Mouth.

**Bacillus buccalis maximus.**—This consists of isolated bacilli or filaments, more frequently of bundles. The filaments of the length of 30—50 micro-millimetres (Fig. 13 of Miller, stained with the acidulated solution of iodine) are distinctly articulated.

The isolated bacilli or articulations are from 2 to 10 micro-milli metres; their thickness is from 1 to 1·3 mm. Of the primary fungi of the mouth this is the greatest; all its parts cannot be coloured with iodine (Fig. 14 of Miller to be compared with our Fig. 2, *m*, *m*, *n*, *n*, *n'*, *q*, *r*, and with Fig. 5, *a*, *b*, *c*, *d)*. It is not detected in the dental tubuli, probably because it is too big to penetrate there.

Miller holds it to be of a different species from *Leptothrix innominata*, from its size, and through possessing segmentations or knots, and not bending in zig-zag; and also on account of its very strong behaviour towards iodine re-agents. But these points, very feeble in themselves, are not even constant. Take, for example, the specimen drawn in Fig. 9, *d*, in which the filament of *Leptothrix* (fertile filament not segmented) springs from a portion of the chain which rose on the top of a large, knotty filament. If these large filaments appear in stumps or in separate networks it is owing to the growth of superior vegetation and the subsequent rubbing off of the latter by friction.

I shall presently show that *Leptothrix* living in the water is absolutely identical with this *Bacillus maximus* of Miller.

I have, however, to observe that very distinct segmentations exist sometimes, even in the most slender filaments. In Fig. 5, *g*, I observed an opaque segment, deeply coloured in the middle, with two clear, pale segments at the ends. This filament, compared with bacillus *d* (same figure), hardly gives the thirtieth part of its thickness. Miller himself, in his previous work of 1883 on *Leptothrix gigantea*, describes small rods and cocci within the sheaths of filaments of the said *Leptothrix* (Figs. 2, 3, and 4 of relative plate). In Fig. 5 he even drew a cumulus of small rods, sprung out of the filaments through bursting. The very slender filaments drawn in Figs. 2, 3, and 4 are also articulated.

**Leptothrix buccalis maxima.**—The same author is not certain whether this is a really separate species or a younger form of the same *Bacillus buccalis maximus*. The only difference consists in its resisting an iodine reaction, and in the greater distance of its articulations.

**Jodococcus vaginatus** (Fig. 15 of Miller, reproduced in our Fig. 7) is found rather plentifully in uncleaned mouths. Miller found it only in two children. It is formed of rom four to ten cellules or nuclei, seldom more, placed obliquely in chain, and having the shape of little flat or round shields, or tetrahedrons. The chain is either bare, as in *p*, *p*, of Fig. 2, or sheathed. The sheath, large, 0·75 mm. (I have found some even larger) is colourless or takes a yellowish colour, with a long iodine saturation. The nuclei are tinged with dark violet. At times the sheath is broken, the place of the nucleus is empty, or it has dropped off.

I shall not repeat what I demonstrated in Section I. with regard to these chains—of their envelope tardily discovered, of the presence on the same of curved diplococci, morphologically identical with the gonococcus of Neisser. I shall only state that, as such forms are found, even of greater dimensions, in urine, and being intermixed with filaments and other forms of *Leptothrix*, their relation with this parasite, which is most widely spread, becomes still more probable. It is, perhaps, the only one which lodges simultaneously in the mouth and in the external genito-urinary mucous membrane.

But, even admitting the *Jodococcus vaginatus* of Miller to be entirely different from the small sheathed chains described by us in Fig. 4, their entity as a distinct species still remains doubtful. The same author, in the quoted work of 1883, described and drew (annexed plate, Fig. 8) some articulations divided in two and in four small cocci, arranged, as in the *Jodococcus vaginatus*, by couples or tetrahedrons. He even found in the pig *whole filaments* of *Leptothrix*, made up with a series of those cocci, as we shall see later.

**Spirillum sputigenum** (Figs. 16 and 17 of Miller and our Fig. 5, *h*).—It is found in every mouth, and mostly upon unclean teeth. We should note this circumstance, as it corroborates our views. Cleaning the teeth destroys, every time, the pseudo-inflorescences or productions by points, from which would rise those Comma bacilli, together with the spindle-like and snake-like bacilli. We shall see, in fact, that the simple act of mastication is apt to destroy those pseudo-inflorescences, so that we must look for them on empty stomachs. Is it to be wondered, then, if their products, and chiefly the *Spirillum sputigenum*, are so scanty in those persons who regularly clean their teeth? Miller goes on to say that in unclean mouths they are numberless.

These microbes are in the shape of small Comma rods, endowed with very quick, screw-like movements. When linked by twos, they form themselves into small snakes, like the letter *s*.

Lewis, as we have already said, identified this microbe with the bacillus styled *Cholerigenus*. However, its presence in the mouth had been detected before, and by a few attributed to fragments of *Spirochæte*. Clark held it to be the cause of caries, for it penetrates the dental tubuli as shown in Fig. 17 of Miller. This author excludes every relationship between the *Spirillum sputigenum* and the bacillus *Cholerigenus*, judging from the culture which for the latter is positive whilst for the Virgula of the mouth it is always negative. However, this is a new confirmation of our views, for, if the Commas in question are real organs or copulative filaments, it is quite natural that, whilst they fecundate the articulations or the spores, they should be incapable of reproduction themselves.*

In addition to the *Spirillum sputigenum*, Miller classifies two other types of curved filaments: one short, massive (Fig. 32), and motile, which liquifies gelatine; the other slender, still, and more curved (Fig. 18). In growing old, it gives rise to a small

* After having completed our work, we found in the *Lancet* of June, 1890, an important article by Dowdeswell, on the Comma bacillus of cholera. He touches on the widely-spread hypothesis that those Commas may be only fragments of spirilla, and then he describes three cycles in their evolution:— *A*, Commas or fragments of spirilla which may or may not end in sporules; *B*, Active cellules, with cilia and amœboid forms; then round quiescent cellules (sporanges) ending in minute sporules; *C*, Active filaments ending in sporules of the first generation. The author, however, has not succeeded in reproducing, *by any method*, the normal Commas, vital and reproductive for themselves. He affirms that cholera bacilli are to be found, normally, in the large intestine of the guinea pig, and that the subcutaneous inoculation of a large number of such bacilli really produces choleriform symptoms in that animal; but it is not a true cholera infection. Considering the high temperature that accompanies it, it is a true septicæmia. The fact, then, that the author nas found forms of *Leptothrix* in the Comma bacillus cultures, goes still further to support our views. Dowdeswell, Note on the "Morphology of the Cholera Comma Bacillus," in the *Lancet*, 1890, Vol. I., page 1419—23.

chain of cocci. The author has not detected any spores. Although this microbe grows in the gelatine, it does so in a different manner from the cholera bacillus.

**Spirochæte dentium** (Fig. 20 of Miller and our Fig. 6, *c*) is not found in the decayed teeth, but on the edge of the gums, together with the *Spirillum sputigenum* (affection of the gums). It exhibits spires 8·25 mm. long, of various thicknesses. The more slender ones hardly become coloured. The author himself doubts whether the slender spires constitute a separate species, because he considers the largest amongst them to be akin to the *Spirillum sputigenum*. Their development is probably unknown. "We know little or nothing (he says) about the vital conditions and their manifestations, such as fermentation, pathogenic action, etc."

I shall observe that, amongst the materials which came under my examination, I have constantly found these *spirilla* or *spirochæta* with the *Leptothrix*. In the former Memoir on relapsing fever, I spoke of Spirilla and Spirochæta, which, together with filaments, small rods, and other portions of *Leptothrix*, are produced in infusions of potatoes. In a later paper upon a Diplococcus analogous to the Gonococcus of Neisser, in a case of Carcinoma of the bladder, I mentioned the striking *Spirochæta* that are found in the sediment of bottles filled with the stale water. Together with Zopf, I have retained the name *Spirochæta* for those spirilla which bend in the middle, and thus mark a sort of transition between the so-called *Spirillum* and the *Spirulina*. I may add that, in the sediment of the bottles, the *Spirochæta* surround the tiny islands or lumps of a stringy microphite, which, in its natural state, resembles the filaments of the *Leptothrix gigantea* from the dog, especially the more slender ones, drawn in Figures 2, 3, 4 in the already mentioned article of Miller. On the other hand, the intertwined filaments of that sediment, being coloured with aniline, resemble entirely the lumps of the *Bacillus buccalis maximus* of Miller, after Fig. 13 in the last work of this author. According to Zopf, *Leptothrix* is a microphite that lives in the water.*

* Zopf, *Die Spaltpilze*, Breslau, 1883, page 80.—Vicentini, On a diplococcus analogous to the gonococcus of Neisser, found in urine, in a case of Carcinoma of the bladder—Vol. XLIII., *Degli Atti Della R. Accademia di Napoli*, 1889.

I have already stated that, in the midst of those masses of filaments of the sediment in the bottles, there are preserves of Vorticellas.

Now, it is not improbable that the *Spirochæta* rise there from the more slender filaments of the *Leptothrix gigantea*, and in the mouth from those of *Leptothrix buccalis*, as I shall show later; and that the *Leptothrix gigantea* and the *Bacillus buccalis maximus*, or *Leptothrix buccalis maxima* of Miller, may be the same thing. I have remarked that, in the sediment from the bottles, the vorticellas and filaments are never wanting; whilst the *Spirochæta* are found there only in summer, and then in brisk motion. Hence, we may infer that the temperature may greatly influence the production of the Spirilla and the Spirochæta, as Miller had argued from the cultures, and as their abundance in the mouth would prove.

I shall refer later to the Spirilla that, in certain cases, I have seen accompanying the *Leptothrix* of the prepuce and the urethra.

## Secondary Micro-Organisms.

**Leptothrix gigantea** (Fig. 21 of Miller).—This form was found on the teeth of a dog affected with alveolar pyorrhœa. It was also found on other carnivorous and herbivorous animals. It exhibits a vigorous development; has a bushy shape, like the *Crenothrix*, the stems of which deflect at the top, with cocci, small rods and filaments sometimes alternated on the same line. The stems are polymorphic of varying thickness. Some increase in size towards the top; some are partially or wholly spiral; and others double and twist as in *Spirulina* (Fig. 12 of the plate annexed to the article of 1883).

In this article, published in the *Berichte der Deutschen Botanischen Gesellschaft*, are to be found more particulars upon the microphite in question. The oldest filaments are articulated; some are spiral and mutually twisted at the base (Fig. 1, *E*, *a*). The articulations of the more slender filaments cannot be detected without colourisation. There are sheathed filaments whose internal articulations sometimes evacuate and form cumuli of little rods of different sizes (Fig. 5); others become compressed or swollen, or deviate from the axis (Fig. 1, *A*, *B*, *C*). By staining process we find, particularly in the pig, articulations subdivided into two or four cocci; or groups of two or four, which at times occupy the whole length of the filament (Fig. 8). In the tufts can be seen straight and tortuous filaments. The bending is graceful and extended. These are styled by the author, *Vibrionic* or *Spirochætic* forms (reproduced in our Fig. 8). In the more slender filaments we can observe the transition from the vibrionic to the spirillic form upon a single filament (Figs. 10 and 11). In the spirillic forms of these slender filaments, by means of a proper colouration, the articulations can be detected.

In 1883, Miller suspected that the same slender spires of the *Patina Dentaria* would rise from the fragments of long and slender tortuous filaments (Figs. 13 and 17). In the dental Spirochæta there is no trace of articulations, but in the marshy Spirochæta, through the weak colouring of Zopf, are detected several articulations in every filament (Fig. 20). Hence, then, the author inferred that relations existed between the Spirochæta and other microbes of the mouth. But now, in his work of 1889, he seems to have discarded his former theory of unity of forms.

Miller is unable to say whether the *Leptothrix*, detected by him and by others in the tame herbivora and in the pig, is identical with that which lodges in the mouth of man and of the carnivora. He says that it would be necessary to institute pure cultures. Anyhow, it is important to admit the existence of *Leptothrix*, even in the herbivora. Whilst before it was held that *Leptothrix* lodged exclusively in the mouth of carnivora, Zopf had already admitted its existence in the herbivora.* It is true that it is less frequent in the herbivora; but, if we are not mistaken, it must be taken into consideration that these, and especially the ruminants, are continually using their teeth, so that the parasite cannot thrive there at ease.†

* Cornil and Babes' quoted work, page 135; Zopf already quoted. This author thinks it probable that *Leptothrix buccalis* originated from the external world, especially through water and food. We have already mentioned the *Leptothrix* found by us in the sediment of a bottle of water; but how can we explain the *Leptothrix* in genito-urinary passages?

† Miller, *Uebereinen Zahn Spaltpilz*, *Leptothrix gigantea* (*Berichte der Deutschen Botanischen Gesellschaft*, Heft 5, 1883, p. 221).

**Fungi of the Mouth which may be stained blue and violet with Iodine Colours.**—Besides the *Bacillus buccalis maximus* and the *Jodococcus vaginatus*, Miller points out three others with a striking iodine reaction, viz. :—(*a*) *Jodococcus magnus* (Fig. 22), which colours violet ; (*b*) *Jodococcus parvus* also stains violet ; and (*c*) another *Jodococcus*, which takes the rose colour with iodine, and can be cultivated a first time, but it does not reproduce in a second culture.

**Fungi of the Mouth which can be cultivated.**—Some of these are not pathogenic ; some of a doubtful pathogenic efficiency. In Fig. 23 of the author are depicted several various types of these bacteria of the mouth ; in *a*, *c*, *g*, screw forms ; in *b*, cocci ; in *d*, small rods ; in *e*, a sheathed chain of cocci ; in *i*, a long chain (but these are numberless in the patina of the tongue, so that we believe it to be impossible to separate them from *Leptothrix*) ; in *f*, chains of small rods ; and in *k*, thread forms (to which the same remark applies).

Miller himself considers it a confusion, difficult to clear up without immense labour, as all the bacteria of the external world can form a nidus in the mouth. The author depicts 19 out of the 22 species which he separated in 1885, viz., from Fig. 24 to 27, cocci ; 28 to 29, small rods ; and 30 to 31, still longer rods ; in Fig. 32, a *Vibrio viridans* that liquefied the gelatine and produced a green colouring matter ; a spirillum (Fig. 33) ; long threads (Fig. 35) ; and small chains, partially sheathed (Fig. 34).

We shall omit seven other types obtained from simple cultures.

Miller afterwards points out the investigations of Vignal, which he finds fit and accurate. Such investigations led to the cultivation of 17 species of fungi in pure cultures ; some identical with known fungi ; others unknown. The more frequent was *Bacterium termo* ; then come *Bacillus ulna* ; then the Bacillus of potatoes ; three other unknown Bacilli ; the *Bacillus subptilis* ; then the *Staphylococcus pyogenes albus* ; then the *aureus* ; five other unknown Bacilli ; and, finally, the *Leptothrix*.

From that view, there would result for Vignal a great preponderance of bacilli ; for Miller, on the other hand, of cocci. Miller attributes this divergence of results from Vignal having used the gelatine, on which not all of the above mentioned microbes thrive,

Black, through his own researches, has found in the contents of the mouth, *Streptococcus continuosus*, *Staphylococcus medius. Staphylococcus magnus*, *Coccus cumulus minor*, and *Bacillus gelatogenes.**

We have not been able indeed to convince ourselves that, from those experiments of cultures, a basis may be established for the identification of the species of bacilli or bacteria, owing to their unlimited polymorphism, according to the various nourishing media and the different conditions of their surroundings; so that, wherever the decisive proof of the fructification is wanting, the results of the cultures for creeping or immersed vegetation cannot be freely accepted as the same. In doing so we might run the risk of multiplying the species and the true or supposed pathogenic properties of bacteria, *ad infinitum.*

Different particles or cellules of the same organism may, in fact, need various nutrient media; they may liquefy certain substances, and separate others of a very different nature; as, for instance, the bony corpuscle, the hepatic cellule, the blood corpuscle, the Renal Epithelium, etc. The poison of insects or snakes is not diffused throughout their organism, but it is secreted by special glands. Likewise, in the higher vegetation, the leaves, the roots, the buds, the seed, etc., often contain principles or alkaloids totally different from the other parts. And the same particle or cellule can assume various forms according to its stage of development, its surroundings, or nourishing pabulum.

Furthermore, the virulence of bacteria may decrease or disappear in certain cultures; increase or reappear in others. Now, if the virulence constitutes for itself a truly specific character at every step, we shall have to admit, in those microbes, changes of species; and thus, in order to defend the true and well-thought pleomorphism, we must necessarily fall into an illimited and unlikely one.

However, referring to the six species of micro-organisms admitted by Miller in the cavity of the mouth, we, with due respect for the author, cannot abandon the opinion (which, although old, is not ours) of the fundamental unity of all these forms, and their derivation from a single species, the *Lep-*

* Miller, *Die Mikroorganismen der Mundhöhle*, etc., 1889, pp. 43—75.

*tothrix buccalis* of Robin; of which, in the following section, we will give our description in its principal biological phases. At most, some doubt might be entertained in regard to the Spirilla or Spirochæta, whether to hold them as a species, or belonging to a distinct species; but their relationship with *Leptothrix* was recognised even by Zopf, who, on this point, wrote: "In certain cases, the filaments (of *Leptothrix*), either on a given part or in all their length, take a spiral shape, and the fragments of those fibres form the *Spirilla*, *Vibriones*, and *Spirochætæ*."*

Miller himself in mentioning his studies, made in 1883 upon those of Zopf, also admitted the derivation of the *Spirochætæ* of the human mouth from the twisted threads of *Leptothrix*, of which he gave a representation of the fragments in the figures 13 to 20 of his plate; and, in the description of the branching filaments of *Leptothrix gigantea* of the pig (Figs. 6 to 8) and of the sheep (Fig. 9 to 12), he represented by the side of straight filaments other vibrionic and spirillic ones.†

We have besides, by our observations, pointed out that the *Spirilla* or *Spirochæta* are constantly united with *Leptothrix*, even in the infusion of potatoes and in stale water (in a warm atmosphere). Later on, we shall see that they are also found in certain urinary mucous flakes, always accompanied by vigorous fructifications of *Leptothrix*.

### Pathogenic Fungi of the Mouth.

We conclude this short extract by summing up the views given by Miller in Chap. IX. of his work upon the experimental infections produced through the inoculation from certain microbes of the mouth, or accidentally lodged in it.

The author begins by reminding us that the venomous property of saliva was long before known when, in 1873, Wright and Senator used it for the purpose of inoculation with deadly result. It was suspected that the poisonous effect depended upon a chemical cause. Moriggia and Marchiafava, in 1878, inoculated with the saliva of children dead from rabies. The first who

* Zopf, *loc. cit.*, page 80.

† Miller, Article *cit.* 1883, pp. 223—24.

attributed to bacteria the venomous action of the saliva or sputa were Raynaud and Lannelongue, in 1891. The rabbits that were inoculated with the saliva of a boy who died of rabies, died; whilst the inoculations with the blood or the buccal mucus were harmless. Pasteur saw two rabbits die after inoculation with the same saliva; and the cultivated microbes exhibited the shape of dumb-bell bacteria in a gelatinous capsule. He then believed he had discovered the cause of hydrophobia; but Cohnheim, having noticed the rapidity of the infection, qualified it for septicæmia.

After these first experiments, Vulpian produced the same infection by inoculating with the normal saliva; and, in the blood of the inoculated animals, Rochefontaine and Arthaud detected microbes quite identical with the above-named. Sternberg and Claxton confirmed the observations of Vulpian; but Griffin demonstrated that the saliva of the parotides, gathered separately, is harmless; so that the infection is to be attributed to secondary products of decomposition, formed in the buccal cavity. Gaglio and De Mattei held the same view, and noticed that the saliva became harmless after boiling.

Fraenkel in 1884 confirmed anew the observations of Vulpian. He found that dogs were more receptive, the mice less so, and still less guinea-pigs. Dogs, pigeons, and fowls were refractory.

Miller obtained identical inoculations with the excretion of a woman affected with micosis of the tonsils. He separated afterwards from a decayed root five fungi, of which one was distinctly pathogenous.

Klein and others studied the venomous action of groups of microbes of the human sputa, so that in late years pathogenic properties were gradually attributed to a considerable number of bacteria.

**Pathogenic Fungi not Cultivable.**—The author repeats here what has already been said of the primary microbes of the mouth, to demonstrate that *Spirillum sputigenum* and *Spirochæte dentium* are especially not cultivable. Kreibohm found in the patina of the tongue two pathogenic bacteria unfit for cultivation in any artificial medium whatever. Miller, with fungi from a gangrenous pulp of the teeth, obtained a gangrenous product for re-inoculation.

**Pathogenic Fungi Cultivable.**—(*a*) *Micrococcus of the sputum Septicæmia* (Figure 96 of Miller).—This is the same bacterium that Pasteur and others obtained from healthy and pathological sputa. Klein was the first to cultivate it. It results in oval cocci, diplococci, and capsulated chains identical with *Pneumococcus*. Subcutaneous injections with its cultures produce septicæmia and death in 24—36 hours. Even the blood of animals inoculated is totally infected; but the infection, once overcome, is contracted no more. Its virulence is attenuated by cultures in milk. This bacterium (continues Miller), *lodging constantly in the human mouth*, passes from there into the pulmonary tubes; so that the uncleanliness of the mouth would be the real predisposing condition to pneumonia, in so far as it allows the diplococci in question, deposited there from the air, to multiply. From those views to ours, to the entirely secondary propagation of such bacteria in the pulmonary products, there is but one step. (See Section 4 of Memoir on Whooping-cough.)

(*b*) *Bacillus crassus sputigenus* (Fig. 97 of Miller, from the blood of a mouse).—This bacterium was found by Kreibohm once on the patina of the tongue and twice in the liquids of the mouth; it is longer than it is broad, but often shorter in its younger articulations; resists Gram's method; sporifies through heat. Mice, inoculated even with small quantities, die in forty-eight hours, exhibiting myriads of bacteria in their liver and blood. A copious injection of it into the veins kills rabbits and dogs in from three to ten hours from gastro-enteritis.

(*c*) *Staphylococcus pyogenes albus* and *aureus*, and *Streptococcus pyogenus*.—These bacteria, held as promoters of suppuration, are brought from outside into the mouth, where they seem to find a fit soil. Black, upon ten specimens of buccal liquids and scrapings from the tongue, found seven times *Staphylococcus pyogenes aureus*—four times the *albus* and three the *Streptococcus*, which he believes with a careful search can always be found. Vignal never came across the true streptococcus; seldom found the two staphylococci. Netter, upon 127 specimens, found only seven times the *Staphylococcus pyogenes aureus*. Miller also rarely found these micrococci.

(*d*) *Micrococcus tetragenus* (Fig. 98 of Miller).—This micrococcus, relatively frequent, was first found by Koch and Gaffky in the cavities of a consumptive subject; it is more frequent in tuberculosis. Biondi found it in three cases out of five. It is in the shape of cocci disposed two by two or four by four; with gelatinous matter interposed, it does not liquefy the gelatine. White mice and guinea pigs inoculated with its cultures die in from three to ten days; those inoculated with sputa perish in from four to eight days. Their blood and their organs are infested with micrococci.

**Fungi of Biondi.**—This investigator has isolated from the mouth five different pathogenic bacteria, including the *Micrococcus tetragenus* above-mentioned. The four others are:—(*e*) a *Bacillus salivarius septicus*, rather frequent; (*f*) a *Coccus salivarius septicus*, obtained in a single case only from a woman affected with a serious septicæmia in her confinement; (*g*) a *Streptococcus septicus*, similar to that of erysipelas, *phlegmon* and puerperal *Metritis*; (*h*) a *Staphylococcus salivarius pyogenes*, found once in the sputum of a scarlatinous quinsy.

**The Fungi of Miller.**—After many cultures and inoculations from buccal liquids and dental gangrenous pulp, Miller observed more serious effects from the inoculations of the pulp, even in comparison with inoculations of cultures of the same pulp. The effects were varied in different animals and according as the inoculation was made under the skin or the peritoneum cavity. The pathogenic fungi isolated by the author were:—(*i*) a *Micrococcus Gengivæ pyogenes* (Fig. 99 of Miller), obtained from alveolar pyorrhœa, cocci that did not liquefy the gelatine; (*k*) a *Bacterium Gengivæ pyogenes* (Fig. 100 of Miller), found with the former, in the shape of bacteria four times longer than broad, apt to liquefy the gelatine; (*l*) a *Bacillus Dentalis viridans* (Fig. 101 of Miller), obtained from the decayed roots of teeth, in bacteria of irregular forms; (*m*) a *Bacillus Pulpæ pyogenes* (Fig. 102 of Miller), obtained from the gangrenous pulps, in bacilli, frequently irregular, isolated, linked together, or in small chains, even with eight articulations, apt to liquefy the gelatine.*

We cannot further describe these culture experiments. We

* Miller, *loc. cit.*, pp. 199—220.

shall only remark that the pneumococci, or theexciters of salivary septicæmia, being constantly found in the contents of the mouth, are to be held, in our opinion, as one of the forms or phase of the microphite or microphites of the mouth (zoögleic phase of Billet). With regard to all the other bacteria we have just mentioned, it remains to be observed whether they are accidental—*i.e.*, proceeding from without—or normal, although modified in their properties by variable conditions of pabulum or surroundings; and whether their numerous varieties are perhaps more apparent than specific.

As regards the experimental inoculations, we shall simply notice the fact that they introduce, under the skin, in the cavity, or in the circulation, all at once, myriads of bacteria. A single centigramme of culture or of polluted material may contain, on an average, twenty-five billions of bacteria, as we have elsewhere demonstrated; and twenty-five milliards inoculated in a mouse are as fifty or a hundred trillions (Italian measure) inoculated in a man. Is it wonderful if local or general effects may be produced from the sudden irruption of such a mass of parasitical germs, even though in themselves innocuous? Nevertheless, that is not the real question; the real question is whether the different kinds of bacteria (probably belonging to the same species) have the power of attacking, *sponte* and on their own account, the human economy; and whether, even penetrating it from their media, in an infinitesimal number (as is the case in the ordinary infectious diseases), can they develop in the human species the same effects following upon the inoculation of their elements on the brutes?

In other words, one is the receptivity through the natural passages and another through the continuous solutions; one is the slow, successive, and external action of a few germs, another that of the sudden and internal mass of germs. Finally, one is the receptivity of this or that animal species, and another that of the human species. And all this is irrespective of the other more or less questionable points, and especially of the query: If the morbific action of the inoculations depends upon the bacteria, as such, or upon the polluted matter in which they are suspended; or, it may be, upon their products of secretion and decomposition, as is at the present time generally argued.

But, leaving for the present those complex questions, I will proceed to speak of the morphology and biology of *Leptothrix*.*

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§ IV.

## REMARKS ON THE MORPHOLOGY AND BIOLOGY OF THE LEPTOTHRIX BUCCALIS.

### The Four Phases of Leptothrix and particularly the Fructifications by Ears (Spicæ).

First Phase.—The first phase of the *Leptothrix* is that of a Bacterium or Bacillus, already described in the Memoir on Whooping-cough and in the first paragraph of the present one. This phase is common to all the other species of bacteria, but which (at least in *Leptothrix*) does not represent its whole cycle of life, but only its primordial stage of immersed vegetation, or a vegetation destined to propagate in liquid or semi-solid substrata (media).

Second Phase.—Examining the cuticle of the tongue, stained with gentian violet, we find there by preference the forms of the second stage of life of this parasite—namely, that of chains, bundles, and masses of intertwined filaments. We have already said that in these preparations the buccal epithelia predominate; isolated filaments are found there, as well as many specimens of large dumb-bell bacteria of the type *p p*, and *p'* (Fig. 2), and conspicuous masses of diplococci. These preparations show that the large dumb-bell bacteria are derived from the diplococci, the two original cocci linking together. The degrees of transition to be observed there are many. The same chains are often surrounded

* Two fresh works were lately published on this argument: one by David (*Les Microbes de la bouche, precédé d'une lettre-préface de M. L. Pasteur*), a simple compilation; the other by Billet (*Contribution à l'étude de la Morphologie et du développement des bactériacées*), the result of original researches on the natural history of four species of micro-organisms (*Cladothrix dichotoma*, *Bacterium Balbiani*, *Bacterium osteophilum*, and *Leptothrix parasitica*, a parasite of sea-weeds). We shall notice these works later on; we may at once say that not even these authors hint in the least to the SUPERIOR PHASES of *Leptothrix buccalis*, after our type or other's.

by similar masses of diplococci; even the entanglements of the type *u*, *u*, are not wanting (Fig. 2).

The chains belong to types *c* and *d* (Fig. 2), but are generally longer, thick, branched off in bundles of 10 or 15, which take up to three visual fields, and exactly represent the disposition of the bundles of filaments. In the same chain small diplococci may alternate with elliptical bacteria and medium-sized dumb-bells—all perfectly equal, which form the largest number.

In these we have been unable to find fructifications, so that this second phase may be held as analogous to mycelium, or the vegetation, of fungi, partly immersed, partly creeping.

Third Phase (*Incomplete aërial vegetation*).—This is generally met with in the tartar of the teeth. Its predominant elements are the large filaments, often very long, bent, and re-united in bundles, taking a brilliant colour with iodine; and the stumps. I shall not describe these well-known forms, having already done so in speaking of the primary and secondary species of Miller.

I would rather point out some features, noticeable in a certain number of these filaments, when I have the opportunity of finding them isolated (especially in the unstained preparations), such features not having been considered by previous authors.

The first is a division into two or three branches (Fig. 9), which I think must be taken for a kind of radical system of this microphite, for divisions are never detected towards its apex; and, at its opposite end, a swelling or head is noticed, which is a proper form of points twined upwards (as in *b*). In *a* the filament is broken towards the top and exhibits two long roots; in *b*, there are three hood-shaped roots. Both appear to be swollen at their ends, like *haustoria*.

What can be the use of these barbs or roots, unless to obtain a firm foundation in the ground? And to what purpose is this firm foundation, unless to support higher forms of vegetation?

The other feature is that of the swelling, which we will call *apices* or heads, being found at the top of the respective filaments, as in *b* and *c*, and these are of varied form, at times containing a kind of nucleus. There are others which might be styled knotty, being found along the filament; of these we give no drawing, as they are similar to the preceding ones.

With the above-mentioned stumps are intermixed almost all the forms of bacteria, bacilli, spirilla, etc., besides a large number of very varied small chains, after the types *c*, *d*, *k*, *i*, *x*, and *x* (Fig. 2).

The larger size and the facility to colour of these masses and stumps, with regard to fertile filaments, would depend, I think, upon a very natural cause. The friction, removing the points of the filaments and the fertile filaments, seems to impart a greater development to the remaining stems in the sense of thickness as, for instance, it happens in the pruning of trees, through the retrocession of the ascending sap.

In the present case, the retrocession of the germinal matter would be the cause of the consecutive increase of the sheath and of the gemmules contained in it. Thus might be easily explained the appearances, so various, of the stumps and their fragments, compared with the fertile filaments; appearances which induced Miller to make of residual filaments two distinct species, under the name of *Leptothrix buccalis maxima* and *Bacillus buccalis maximus*, in opposition to our fertile filaments, which for Miller constitutes a third species, the *Leptothrix innominata*.

It remains, then, to be seen whether the longer filaments, sunk down and intertwined, of *Bacillus buccalis maximus* (Fig. 13 of Miller) may not concur, with the above described chains, to form that kind of mycelium or creeping vegetation, upon which is based the aërial vegetation of this microphite.

From the tiny islands of the stumps in question, prepared in the manner already described in Section 2, spring at last the fructifications. The bigger filaments, that we would call woody, gradually become thinner and pale, showing in their interior countless granules or parietal gemmules. These are the fertile filaments. They may spring either from the proper filaments, with continuous contour, or from the little chains after type *d* (Fig. 9), which are seen occasionally on the top of those filaments; and in this last case, instead of a gradual thinning, may abruptly pass from the chain to the fertile filament, as shown in the figure.

It is by no means uncommon to find parietal gemmules, as Robin mentions the circumstance, and gives a first drawing in Fig. 2*h* of his volume of 1853, magnified to 800 diameters.*

* Robin, *Hist. Nat. des Végétaux, etc.*, 1853. (See Bibliography.)

FOURTH PHASE (*Completed Aërial Vegetation: Fructification*).—In Figs. 10—13 are reproduced four specimens of fructifications, taken from the *patina dentaria.* In Fig. 10 are seen three isolated "ears" *(spicæ)*, stained with gentian violet; in Fig. 11, a tuft, caught as the bird flies, stained with weak methyl violet; in Fig. 12, a part of a more vigorous tuft, seen in profile, stained with weak fuchsine; and in Fig. 13, two fructifications, set on the same tiny island of *materia alba,* stained with solution of iodine. The Figures 10, 11, and 13 are drawn magnified to 1,250 diameters, and the Fig. 12 to 850 diameters.

The fertile filaments are at times partially straight, as in Fig. 10; at times bent (even occasionally tortuous, as in Fig. 13), and are sometimes wanting, because the fructifications have been carried away by mechanical force, although grouping together, as in Fig. 12. In Fig. 10 are seen, in *a, a,* the gemmules of reserve adhering to the walls; in *b* are found the little spores properly lodged; but in *b'* we notice only five, the others having dropped. In *c* the penultimate articulations of the stalk appear older and woody; the last is granular, like the two articulations on the apex of the younger filament, *d.*

The sporules, very small, round, brilliantly coloured, show themselves in three vertical rows in all the specimens without exception; *i.e.*, they never appear in two rows, whence we may infer that they are, in fact, disposed in six longitudinal lines (or series), for, if they were only in four or five, they should, at times, exhibit only two visible rows.

In turning the micrometer screw, the cylindrical relief of the "ears" appear manifest, for at a higher focus the sporules of the middle row are better seen, and at a low focus those of the lateral rows. In several specimens, especially when coloured with picric acid, we can detect the prolongation of the stem into the interior of the ear; but it is not so with aniline colours, which render the viscid substance (in which the sporules are suspended) opaque, staining it with a weaker tint. But if, in the preparation, we substitute glycerine for water, the stalk, a long time afterwards, reappears distinctly in all its length.

At any rate, the movements given to the preparation with the thumbs show that the stalk and the "ear" form a continuous whole; then we observe the "ears" on the top vibrating like the ears in a field of wheat.

It may be objected that the sporules are not immediately grafted on the stalk or fertile filament, but are distant from it; it must, however, be taken into consideration that the distance between the sporules and the internal filament is not, in fact, greater than that which is constantly seen between the articulations of the single chains of bacteria, which, as it is scientifically ascertained, are generated one from another. Perhaps a connection exists between them, through threads or ligaments, not discernible by the optical power at our command.*

We cannot even suppose that the sporules are simply adhering to the filament through external action (due to the lowering or sinking, so to speak, of the filaments themselves into the granular masses), because a simple mechanical incrustation would not explain the regular straight line of the sporules in six longitudinal rows, nor their perfect similarity of volume and shape.

The incrustation would bring a medley of cocci and bacteria, of varied type and size, irregularly mixed up. Besides, we shall observe productions of these "ears" (and even more conspicuous) in the sputa, without any trace of bed or granular mass on which the filaments might have become incrusted from the outside.

The length of ears is frequently considerable, especially in those detached from the matrix, which at times, with their stem, occupy all the visual field. The more conspicuous specimens (Fig. 16) have been found in the pulmonary sputum, where the

* When we presented this Memoir, we promised in a note to resume the study of ' *ears* " in detail, with more powerful objectives. Now Messrs. Bézu, Hausser, and Co. have succeeded in constructing a new objective with homogeneous immersion, of 1/25th inch. With this power we have been able to detect, in the most evident manner, the peduncles or engrafted threads of the sporules on the principal stalk. With the new grand model (No. VII.) of the same opticians, furnished with an Abbé condenser of the numerical aperture, 1·40, by using oblique light, and by turning the stage of the microscope, or substituting a dark diaphragm for the ordinary one, the disposition of the sporules in six longitudinal lines is fully confirmed. We shall treat this point again later; but from this moment we think it is better to give the name of "bunches of grapes" instead of that of "*ears*" (to indicate the fructifications of *Leptothrix*), as they really are such.

longest were one-sixth of a millimetre high, equivalent to the thickness of a thin cover-glass, as in the Figs. *f*, *g* of the figure.

The colouration of the ears in question with carmine, protracted even for weeks in a watch-glass, gave no satisfactory results. With methyl violet the specimens appear less transparent; in other respects, they are like those treated with gentian violet. In Fig. 11 one may be observed, in which the *ears* are in the direction of the visual axis. With fuchsine the most fine and brilliant colourations are obtained, as shown in the specimen caught in profile, represented in Fig. 12, after being immersed for a few minutes.

In the uncoloured preparations, the *ears* are hardly or not at all discernible, so that they could not be detected by him who should ignore their existence; the sporules being, in their natural state, transparent and nearly invisible; but these preparations are helpful to him who knows them, in so far as they aid to detect, on the outline of the mass, a larger number of ears which have not been spoiled or carried away by the manipulations, or hardened and consequently become brittle.

Anyhow, the flexibility of the ears is preserved even in the colourings with the solution of iodine, not acidulated, or with picric acid. The sporules appear distinctly with the solution of iodine (Fig. 13); but with picric acid the colouring is diffused and pale, although the pale tint is useful in its turn in disclosing at one time a larger number of fructifications, without rendering any of them opaque.

Here we shall stop awhile to consider the great affinity of those sporules through the aniline colours; which affinity brings them so closely to the tubercular bacilli as to make us suspect that the single articulations of such bacilli proceed from the sporules, which, having dropped, transplant themselves into the buccal epithelia and the air-passages, and grow like beaded forms. We were led to that supposition from the following fact:—In the colourings with fuchsine of the *patina dentaria*, which last a very short time, the central portion of many masses remains uncoloured. How great was our surprise in seeing, some time afterwards, in those uncoloured areas, minute red lines, perfectly similar to the tubercular bacilli! Then those coloured lines gradually increased, and we saw the summits of ears developing, as mounds, brilliantly coloured, in a clear area. This fact is to be attributed to the subsequent action of the colouring matter.

Besides the bacilli of Koch in rosaries, we have others in small rods or filaments containing granules or vacuoli, as in Fig. 1 of the work of Cornil and Babes, etc. These rods appear more clearly when stained by Gram's method—viz., with double colouring, first with gentian violet and then with solution of iodine. Now, the fragments and slender stems of *Leptothrix*, in the *patina dentaria*, behave likewise. If the solution of iodine should be used first, the successive colouring with gentian violet does not succeed; but if we colour with violet, as usual, mounting the preparation when fresh, and then introducing by capillarity the solution of iodine, the violet colouring becomes dark blue and the small rods of *Leptothrix* appear very clearly.

On another occasion I shall treat those questions relating to the finding and colouring of the tubercular bacilli.

### Productions by Points (Male Organs?)

I have already said that such productions or pseudo inflorescences are scanty, so that we must seek them patiently in the preparation. They have, however, the advantage of showing themselves clearly, even without colouring. It is absolutely necessary to examine the *patina dentaria* of persons who have fasted and who are not in the habit of cleaning their teeth, otherwise we may not find these productions. The preparation should not be pressed too much, in order to avoid the disintegration of the specimens. They are easily recognised with an objective, No. 8 of Hartnack. The above-mentioned difficulties indicate why such forms have not been considered by previous investigators.

In Fig. 14 are shown three of these productions in the natural state, taken from the *patina dentaria*, and magnified to 690 diameters. They are formed of one or more internal stocks and a considerable number of points which surround them, like the horsehairs of a cylindrical brush. In *a* the production rises upon a multiple stalk, formed of a bundle of filaments apparently broken off. The stalk itself is crowded with small points near the trifurcation; the three small divided branches bear longer points up to the top; but the right one is also subdivided in two secondary tiny branches. In *b* is drawn an isolated pseudo inflorescence, formed of small and medium points, like the small branches of the preceding specimen. In *c*, on the contrary, is seen a production broken on the top, formed of small, medium, and large points; the small points similar to the preceding ones; the large ones totally similar to the pointed and spindle-like bacilli of type *e* (Fig. 5), of which, for comparison's sake, we have reproduced one in its natural state in *d* (Fig. 14), magnified to 1,250 diameters. Most of the small points resemble the Virgula bacilli of type *h* (Fig. 5); some, however, look like the spermatozoa of type *h'* (same figure), having at one end a kind of small head, according to the degree to which they are magnified.

In Fig. 15 is seen a portion of a small branch, coloured with the solution of iodine, magnified to 2,500 diameters. *Points* of various size and shape are set in it, which, like the central rod, contain clear spaces or uncoloured empty ones. If it were not too bold a supposition, I should be inclined to suggest that the three small *points* are not adhering to the central rod, but are *fulfilling* their functions upon it, and perhaps even upon the bacillus which adheres to it lower down—a *quid simile* with the act of fecundation.

I repeat that it is not my intention to pronounce on the nature of these productions by *points* or pseudo-inflorescences, that being a subject for micetologists. I have only wished to describe facts resulting from my own observations. However, I cannot help noting the very extraordinary mobility exhibited by those *points*, large and small, dropped from the stalks or branches above described.

Those movements are better detected in uncoloured preparations, mounted in simple distilled water, in which they are preserved longer (even from day to day), especially in the minute *Virgula* bacilli; so that I suggest to those who will verify this part of my observations, not to use any tint at all. The stains used, particularly the aniline colours, seem to have a poisonous effect upon those bacilli, or at least exert a lethargic action on their motility. In the largest or spindle-like bacilli of type *d* (Fig. 14) the movements are those of translation, so that they fully justify the name of *Bacillus tremulus*, given to them by Rappin, if that name does not imply a separate specific entity. We notice that those bacilli vibrate not only sidewise, like fishes, but flash here and there with a rapid translatory motion, dashing first upon one tiny island and then (after a pause) upon another, or disappearing from the visual field. It is impossible not to recognise in those motions the character of vital movements, either voluntary or teleological; and excluding the first hypothesis, which would lead us to the *animalculæ* of Leeuwenhoek, Ficinus, and Klencke, there only remains *one external purpose* to this bacilli, which is directed to the performance of one function. That function can only be an important one, considering that those motions are the highest and most persisting manifestation of life exhibited by buccal microbes. Now, what more important function than that of fecundation? *

The movements of the *Virgula* bacilli of type *h* (Fig. 5) are generally undulating and serpentine; but they have also a translatory one, as we have suggested before.

In conclusion, we may suppose that those points, or so-called bacilli, perform a function similar to that of *spermatia* or *antherozoids* of various fungi and sea-weeds (ALGÆ). In such hypothesis the absolute sterility of their cultures would be wholly explained, being quite natural (as before suggested) that simple male organs should not be able to reproduce by themselves alone. It remains to be seen whether these productions *by points* have any relationship with the bacillus *n* (Fig. 2 to the left below), a large bacillus veined through its length, which contains, as in germ, slender and short lineal bacilli towards the inferior end, so that we should be inclined to compare it with a *spermogone* or *antherid.*†

We have hitherto dealt with productions by *points*, in their higher phase; but near these we find other less developed forms,

* In the quoted article of Dowdeswell, to our surprise, we find that he entertains doubts upon the nature (whether vegetal or animal) of the *Cholera* bacillus. See *Lancet*, 1890, Vol. I., *loc. cit.*, p. 1422.

† The spindle-like and *Virgula* bacilli of the mouth closely resemble, in shape and size, the spermatia of the *Sphærella sentina*, of the *Fumago salicina*, of the *Apiosporium citri*, etc. The *spermatia* were considered, since 1877, as male organs, when Cornu noticed that some of them were germinating by themselves, so that their nature would be still uncertain.

supported likewise by one or more internal stems and enveloped in a mass of very minute lineal germs, quite similar to those contained in *n* (same figure). Such lineal germs, engrafted in square on the stalks, resemble spindle-like, serpentine, or *Virgula* bacilli in formation, so that the whole may be taken for the first stage of the pseudo inflorescences, or productions by points, already described. Owing to the difficulty of reproducing them faithfully, we have omitted their graphic representation in the Plate.

## Fructification by Spores (Ears) Reproduced in the Sputa.

I will now deal with the reproduction of the fructification by spores (spicæ) in the sputa. Hitherto we have been unable to obtain cultures of *Leptothrix*, and microbes of the mouth in general, upon the usual artificial soils; and on this point the authors, from Cornil and Babes to the more recent, are agreed.* It will not be inopportune to point out a case of fructification by ears, reproduced in a specimen of sputum, which induced me to make the present communication.

The presence of filaments, isolated or intertwined, of *Leptothrix*, in the sputa, is well known in clinical microscopy. Leyden and Jaffé recognised even *Leptothrix pulmonaris;* and Hunter Mackenzie exhibits a conspicuous specimen taken from tubercular sputum during a time of improvement.† In the sediment of masses of sputa, emulsified with potash, we find not only little rods and filaments, but even numerous tufts of this parasite. I remember also having often found in sputa of every kind freshly stained fragments which I had not known before, and which were portions of ears spoiled in the preparation.

The case which gave the conspicuous specimen, delineated in Fig. 16, was the following :—

* Cornil and Babes, *op. cit.*, p. 135.

† Leyden and Jaffé, *Deutsches Archiv für Med.*, II., p. 488 ; Hunter Mackenzie, *Le Crachat*, etc., trans., Paris, 1888, Fig. 22.

Mr. G. F., 41 years of age, having already been twice attacked by obstinate miasmatic affections, and once by an acute affection of the air-passages (the nature of which he does not remember), fell ill last March with very high fever (from 40·6° to 41·2° in the evening hours, falling about a degree in the morning); with serious dyspnæa (from 45 to 50 breathings a minute), and very rapid pulse (135—145). On the chest it was only noticed that the right apex was less sonorous, giving an indistinct respiratory murmur; little coughing, with scanty and infrequent expectoration; tumour of the spleen by percussion, but probably chronic. The sensorium sound; a little insignificant epistaxis; remarkable bilious diarrhœa. Albumen, one and a-half grammes to every pint of urine, with a few hyaline and granular urinary casts, and increase of ordinary pigments.

In the first days there was a doubtful expectoration, which was thought to proceed from the nose. An eruption of *herpes labialis* appeared (which I remember having deceived me in a case of ileo-typhus). At any rate, the diagnosis of ileo-typhus was given, and three baths were prescribed which gave great relief. On the seventh day the fever suddenly subsided, as generally happens in pneumonia. Dyspnœa remained, only diminished by the quota due to fever; and in thirty-six hours I obtained a small quantity of rusty sputa, a portion of which, nearly free from saliva, I examined, to ascertain whether it proceeded from the air-passages.

The diagnosis was made even clearer when, later on, crackling wheezings and murmurs of friction in unison with the beatings of the heart (pleuro-pericardiac friction of Wintrich) became manifest; these continued up to the sixteenth day of the illness. I have met with this phenomenon on other occasions, in pleurisy or in pleuritic granuloma, but on the left side; here, on the contrary, it was heard on the right upon the line of the sternum, and below towards the mammillary line. Consequently, the grave dyspnœa had been the means of spreading the process to the pleuritic coating of the pericardium, and perhaps to a limited part of the pericardium itself. At any rate, the examples of a great disproportion between the extension of the local and the magnitude of the general facts are not rare, especially when the apices of the lungs are affected with pneumonia.

The following were the elements found in the sputum : a fair number of red blood corpuscles, both isolated and in small clusters ; here and there a great gathering of ellipsoidal epithelia (so-called alveolar), mostly with a coat of granules of myelin, and a few free particles of the same ; many cylindrical and vibratile epithelia of the air passages ; the usual buccal epithelia ; a few slender and short spires of Curschmann ; myriads of uncapsulated pneumococci, generally in vast cumuli ; small well-defined groups of capsulated pneumococci, after type in Fig. 4, *f* ; various other forms of bacteria and bacilli ; filaments, and even tufts, of *Leptothrix* ; groups and large cumuli of spores and sprouts of oidium (*Oidium lactis* ?).

As it is my custom to keep the sputa for whole weeks in order to repeat my investigations, on the seventh day after collecting this sputum I detected in it fragments of fructifications, which I could perfectly well recognise. I then thought of removing a particle of the patina of the sputum, which was adhering to the internal wall of the tube, to that side on which I had always inclined it, in collecting the specimens. There the layer of the mucus was very thin and wet, the tube being accurately closed. This mucous patina, having been adhesive and undisturbed for several days, ought to have preserved nearly the same conditions favourable to the fructification of *Leptothrix*, as seen on the patina of the teeth. That layer appeared to the naked eye opaque and greyish, like a very delicate mould.

The result of that research could not have been more satisfactory, as Fig. 16 shows. It extends over two visual fields (magnified 850 diameters), and the colouring of *ears* with gentian violet is very striking, the particle of the sputum having been immersed for two hours in the liquid stain, then washed and mounted in distilled water. It will be observed at a glance that the bundle of ears has been severed in two groups : in the superior one, *A*, all the stalks are preserved ; in the inferior, *B*, only five are visible. The fructifications or part of them, as well as the missing stalks, were spread here and there over the surrounding visual fields. The inferior group, *B*, has been pressed sideways by the cover-glass, and lies in profile with its base down ; the superior one, *A*, has been pressed down vertically by the cover-glass, and is consequently opened and formed into two, so that it shows five upper stems with the base downwards, and two lower stems, *f* and *g*, with the base upwards.*

In the largest stalk, *a*, the gemmules of reserve are distinctly observed, and these are still better seen in *a'*, where a part of that stalk is reproduced (magnified to 2,500 diameters) after a long saturation in glycerine (see later on). In *b*, the *ear* is partially scattered, so that a few spores remain *in situ*, and the stalk is entirely visible. In *d* and *e*, cumuli of sporules are seen in those points where the *ears* are partially scattered. Then from the concussion the sporules are driven towards the top, leaving bare the stalk near the base. In *c*, the ear is broken, and shows a clear section, but without its superior part.

In the *patina dentaria* I never came across *ears* so long and perfect. In fact, the better kept *ears*, bent down, in *f* and *g*, are the longest found hitherto; as that in *f* measures $166\mu$, or one sixth of a millimetre, which corresponds to one and a half the thickness of a cover-glass, nine to the millimetre. The number of the sporules implanted in these ears (upon six longitudinal lines) may be calculated, at the lowest, to be 720.

We shall have, here, to consider two points. The first is, that a similar specimen cannot be supposed to have been previously formed on the teeth, then fallen in the expectoration with saliva, to be found there second hand, as the fructifications do not rise so high in the patina dentaria, nor could they have been so well kept after the various manipulations. The circumstances under which they were found incline rather to the belief that they had germinated on the mucous film from filaments there deposited, and were being fed from the materials of the sputum itself.

In the present case, we shall not take into consideration whether the presence of red blood corpuscles may have favoured the development of those *ears*. It is certain that they are found more abundant in the patina dentaria of persons suffering from tenderness of the gums, and whose teeth are, in the morning, somewhat besmeared with blood.

The second point is that the specimen in question is sufficient to clear up any doubt whatever about the nature of the above-

* In the Plate, Fig. 16 *B* is erroneously marked Fig. 14 *B*.

described *ears*. Taking only the specimens obtained from the patina dentaria, some doubts might remain, as in it the *ears* are, generally, embedded in the granulous lumps and the entanglements of threads, in consequence of the pressure of the lips or tongue; and we might suppose that they are the mechanical result of that trampling or superposition of minute cocci around the branching filaments, so as to become incrusted with them. But (apart from the consideration that the incrustation cannot explain the perfect similarity of the sporules, nor their constant disposition in six longitudinal lines) the specimen of Fig. 16 dispels any suspicion whatever, as it is there manifestly the sign of a genuine and proper germination; nor, on the other hand, can there be found a particle of resting place on which the stems might have become incrusted.

In support of this view there is the fact that the *ears* acquire, by colouring with aniline, such a solidity that they can hardly be dissevered. The above-mentioned specimen, which, through the accidental crushing of the preparation by a wrong turn of the fine adjustment, was hard-pressed down, exhibits a proof of it. The *ear* drawn in *g* was abruptly removed towards *f*, and made into a spire, but no spores were dropped by the shock, and the *ear* has since gradually resumed its former place. Now this fact could not be explained on the hypothesis of a simple mechanical incrustation.

This preparation has been kept in glycerine, which was afterwards substituted for the aqueous medium. The tint, especially of the *pneumococci* without halo, is very much faded, owing to the diffusion caused by glycerine. The *ears*, after two months of saturation, exhibited a partial decolourisation and withered spores, although the stalks maintained a brilliant colour. The viscid matter has become paler and more transparent, so that the stalks can be detected fully in their whole length; and inside can be seen not only the gemmules, but traces of small knobs, shown in *a'*. By turning the micrometer screw I could even see, although interruptedly, a few sporules belonging to the posterior series or those lying on the slide.

In conclusion, having thoroughly investigated this preparation, it seems to be absolutely impossible to dispute the fructification of those forms of *Leptothrix* and the fertility of their stems. This specimen is at the disposal of anyone who wishes to examine it.

We shall, by-and by, deal with the fructification obtained from urinary mucus.

With regard to the oidium forms found in this sputum, I will say two words concerning their fructifications. I have dealt with this argument formerly (see previous Memoir), and spoken about cultures upon peels of lemons and various other nutrient media; but I have found it more expedient and satisfactory to let the fungi of the sputum germinate in the sputum itself.

The better soil for cultures is that in which spores and branching filaments carry out their immersed vegetation. In order that aërial vegetation should take place, two conditions are required: the first is that the materials of culture (in this case, the sputum) should be moderately dry on the surface; and the second is that the under layer, or the lowest stratum of the sputum, should be kept wet in order to feed the vegetation. Now, both conditions are fulfilled by placing a part of the sputum, impregnated with spores and sprouts, on the bottom of a wine-glass slightly hollow, so that the sputum should sufficiently spread and rise to four or five millimetres. The wine-glass and its cover should be previously cleansed with sulphuric acid, and washed out with alcohol. Through the wide opening of the glass the fructifications can be easily observed and scraped out for microscopical examination. Generally, fructification is completed on the fourth day, by keeping the glass well sheltered in the dark.

The sputum, impregnated with oidium forms, exhibited on the fourth day a simple fructification of *Penicillium glaucum*. Naturally, we do not intend by this to discuss the question brought forward by Hallier—*i.e.*, whether the oidium forms be one phase of the *Penicillium* and *Mucor* kinds.

### Dissemination of the above-described Microbes on the Posterior Organs

Considering now the extended vegetation and fructification of *Leptothrix* upon the dental surface and superposed layers; its abundant germination on the tongue, its constant presence in the epithelia of the cavities of the mouth and pharynx, its very active multiplication in saliva, the countless swarms of analogical forms, quiescent or reproductive, in the whole wide surface of the nasal cavities, and perhaps of its appendages; considering also the extraordinary fertility of this parasite, and its multiplication not only through the fission of cocci and bacteria, but through fructification and budding within the filaments, we may form an approximate idea of the mass of germs or scattered elements which are invading the digestive, aërial, and lachrymal passages.

According to my calculations, not less than from two to three hundred trillions of germs or separated elements are generally present in the mouth and nose, and liable to disseminate the species, at every minute, into the other parts.

In fact, comparing the single sporules of the *ears* with the dumb-bell bacteria of types *a*, *b*, *c*, *d* (Fig. 2), we should have to place nearly eight sporules in two rows upon the surface of a single bacterium in order to cover it. The bacterium is twice as thick as the sporule; consequently, the volume of one bacterium will be, at least, equivalent to that of sixteen minute sporules. Now, in our first Memoir, we have demonstrated that about 25 milliards of bacteria of types *a*, *b*, *c*, *d*, go to form a centigramme in weight; then, for every centigramme of sporules, we shall have to multiply the 25 milliards by 16, which will give 400 milliards of sporules for each centigramme. Calculating now the considerable number of the dropped sporules, that of the gemmules of reserve in the filaments, that of even the most minute granules which constitute the bed of the branching threads and of the clods of *Leptothrix;* calculating the very small lineal germs grafted on the young productions by *points*, we can safely maintain that their extreme minuteness compensates for the larger volume of bacteria enclosed in the older filaments, that of spindle-like, the comma, and the serpentine bacilli, as well as of spirilla and cocci disseminated in every part. Thus, holding the volume of the sporule as the unity of measure, taking the whole patina of the thirty-two teeth as equivalent, at least, to a gramme, we shall have the number of the elements and germs living on the dental patina equal to the product of 400 milliards (in about a centigramme), × 100, viz., 40 trillions. To these figures is to be added the huge mass of germs and elements spread in the saliva, of which a third part may be considered to be formed of microbes: *ut tota aqua vivere videatur*, as, in his time, Leeuwenhoek wrote. The quantity of saliva which continually moistens the mouth is not less than eight or ten grammes; therefore, we have three more grammes of germs and elements, bringing the total up to *120 trillions*.

But we will not further trouble the reader by calculating the other germs and elements lodged on the tongue, in the mucus of the mouth and pharynx, and in the nasal cavities; from which it would result that the figures given above are not at all exaggerated.

It is to be noted that we started with an average of 25 milliards of bacteria per centigramme; a figure twelve times under the calculations of Naegeli—*i.e.*, 30 milliards per milligramme, which would make the mass of germs and elements of nose and mouth upwards of *2 or 3 quadrillions*.

Besides, we must consider that *Leptothrix* lodges, as we have demonstrated, even in the mouth of domestic animals. Now, the mucus, saliva, breath, urine, fæces of these animals, jointly with those of men, constitute, in their whole, an immense preserve. I am led to believe that a great many germs and elements of *Leptothrix* pass into the fæcal matters alive. There is also incalculable diffusion in the air, waters, and soil, especially in populous towns. What wonder, then, that Perroncito has found such a large number of bacteria in the dust of the streets? We may, rather, contend that such a dissemination of buccal microbes constitutes a sort of cloud, which hardly permits external germs to penetrate.

But let us return to the dissemination of *Leptothrix* in the internal organs of the human economy.

With regard to the digestive passages, the number of elements carried there by the saliva must be fabulous, considering that in the smallest drop myriads of them are to be found. Nor is it presumable that their descent into the stomach should prove inactive.

The view that they are partakers in the transformation of aliments is not new; it goes as far back as Hallier, who considered them *necessary* to the transformation, especially of starchy substances, not only in the stomach, but even in the mouth itself.*

* Richter, Monograph quoted in the Bibliography, Part I., Section B 2.

Béchamp compared the transformation of saccharine in the presence of its *microzymas* with that of the fæcula through diastasis.* Warkmann held the same action even for starch, and he conceived that from the bacteria was secreted a true diastatic yeast, destined to transform starch into glucose, although unapt to peptonise the albumenoids.† But, later on, others recognised in buccal bacteria even such a peptonising action, and Miller holds it to be equal to that of the pepsine itself, even without the co-operation of acids.‡

A considerable number of the same germs and elements of the mouth, as well of the nose, is cast into the air-passages at every breath, as the daily observation of the normal sputa shows (see preceding Memoir). Those germs and elements thrive and multiply in the mucus of the bronchi, trachea, and larynx, as the fructification in the pulmonitic sputum, just described, shows; it is evidently a soil fit for the vegetation of *Leptothrix*.

Shingleton-Smith,§ in the quoted paper on *Contagium Vivum*, considers, first, the property that the moistened mucous membrane has of holding bacteria. It does not let them out with the breath, but through the ciliary action of the epithelia, which throws them out with the mucus. This happens with pathogenic bacteria, but, *a fortiori*, it must be so with normal bacteria. The same author quotes the experiments of Tyndal, who found the residual air from the lungs absolutely free from particles which would reflect the electric beam. He quotes as well the observations of Lister and others, who maintained that there were no bacteria in the pus of Empyema with Pneumothorax, the opening on the surface of the lung being very small. This must indicate that the microbes

* Béchamp, *Les Microzymas et les Zymases*, *Arch. de Physiol.*, 1883. The same, *La Salive, la Sialozymase, et les Organismes Buccaux*, etc. (*vide* Bibliography), 1883.

† Warkmann, *Untersuch. über das diastatische Ferment der Bacterien (Zeitschrift für Phys. Chemie*, Bd. VI., 1883*)*.

‡ See Bufalini, *Of the Peptonising Action of Bacteria (Giornale internaz. delle Sc. Mediche*, 1883). Miller, *Ueber Gährungsvorgänge im Verdauungstractus und die dabei betheiligten Spaltpilze* (*Deut. m. Woch.*, 1885, No. 49). The same, *Die Mikroorganismen der Mundhöhle* (see Bibliography), pp. 77—90, where he specially deals with the fermentative action of buccal microbes upon hydro-carbons, albumenoids, and fats.

§ *Journal of Microscopy and Natural Science*, 1890, p. 34.

are kept back by the mucus wetting the larger air-tubes, where the protecting barrier of the epithelia exists; but they cannot reach the slender bronchi or alveoli where the epithelium is evanescent or at all wanting. Thus, the microbes, having reached the border of the smallest bronchi, by means of the ciliary action, they are thrust back towards the superior passages, and carried at last, by the sputum, into the normal or pathological mucus.

But if this happens in normal conditions, it cannot be so in certain pathologic conditions, as in pneumonia, pleurisy, and phthisis. The elements or parasitic germs, in these cases, not only reach the most minute bronchi, but from there they diffuse themselves even to the remotest parts; this may proceed from the insufficiency of the ciliary action, or from an extraordinary concourse of parasitic elements overcoming it, or from the microbes along a denudated tract of mucous membrane.

Förster and Graefe* were the first to write upon the vegetation of filaments or branching threads of *Leptothrix* on the conjunctival or lachrymal ducts; Bizozzero draws a specimen in Pl. V., Fig. 52, of the quoted work. This has been reproduced in our Fig. 6, *d*, and it is natural to suppose that the relative germs originate from the nose.

If now, from the buccal and nasal microbes, we pass to consider those of the external genito-urinary passages, we shall find the same disseminations; but with this difference, that they will not be found so frequently and in such abundance. This depends upon two reasons:—The first is the relative scarcity of parasitic vegetation, especially on the balano-preputial mucus (compared with that of the mouth and nose together); the second is the want of a propelling force, sufficiently vigorous and repeated, capable of pushing, at least in normal conditions, the elements or the germs of the parasites in the urethra or in the bladder. At any rate, we must admit a penetration, on a small scale, by looking at the epithelia of the inferior urinary passages. Such epithelia, even in the most normal and freshly-discharged urine, are found impregnated with bacteria to such a degree that they cannot be attributed to consecutive pollution (viz., after the urine has been

* Förster, *Arch. für Ophtalm.*, t. XV., page 310. Graefe, *Ueber Leptothrix in den Thränenröhrchen* (*Arch. cit.*, t. XVI., p. 324).

passed); but they evidently denote their invasion *in situ* in the bladder or the urethra. Of course, the invasion has happened by a slow, progressive motion of the relative elements or germs, either along the mucus coating, or from one epithelian scale to another, and so forth.

In certain urines, round the small flakes or spires of mucus of the male urethra, clods of *Leptothrix* are met, at times, so large as to occupy the whole visual field of an objective (Nos. 7 or 8 of Hartnack). Such clods are so closely similar to the young clods of the *patina dentaria*, that the most experienced investigator would be unable to distinguish one from another.

Lately, I observed a case of this kind in the urine of a patient affected with chronic pyelitis, having already been subject to several attacks of Blennorrhœa. I placed two of these small flakes in a closed tube with a little of the urine, and for six days I kept them adhering to the internal wall, wetting them gently once or twice a day by inclining the tube. On the sixth day the material of these small flakes, prepared and stained with gentian violet, like the *patina dentaria*, exhibited exactly all the forms of *Leptothrix buccalis*, with the exception of the productions by points (perhaps spoiled in the preparation). Undoubtedly, it contained countless bacilli, both comma and spindle-like, rather small, and in brisk activity; even a fair number of small ears was to be found there. Briefly, all forms of bacteria and bacilli, chain-like filaments, and bundles, described in *Leptothrix buccalis* (except the *Jodococcus vaginatus*) were likewise there; and the staining also showed that not even the *spirilla* or *spirochœte*, mostly in motion, were wanting.

Between these forms of *Leptothrix* and those of the mouth, the only difference is that the filaments, bundles, and chains are shorter and the spirilla extremely slender, but longer. In all other particulars they were perfectly identical.*

* Our present remarks about the multiplicity of the microbes of Blennorrhœa and the presence of *spirilla* in urethral secretion have been confirmed by the research of Legrain, *On Blenorrhœa microbes*. That author found in the flow of gonorrhœa *12 different forms of cocci, 3 distinct forms of bacilli, and 1 spirillic form*: total, 16 forms of microbes (*Archiv f. Dermatologie u. Syphilis*, 1890). Stroganoff has recently dealt with the bacteria of the vagina and cervical canal.—(*Modern Medicine and Bacteriological World*, Oct., 1893, p. 258.)

It would be useful to persevere in this kind of research, and I propose to resume it on the first opportunity, in order to prove the identity of *Leptothrix buccalis* with *præputialis*, and then that of bacteria and bacilli of the air-passages with those of the genito-urinary organs.

We have an argument by analogy for the arrival of the elements or germs of *Leptothrix* in the urethra, in the introduction of more bulky germs, like those of superior fungi, probably of the genus *Penicillium*, either in the urethra itself or in the bladder, of which we have already given an instance in our article upon fungi of the male urethra.

Naturally, the removal, insignificant in healthy conditions, increases in the morbific ones, and that is the reason of the gonococci in blennorrhœa; but we have observed beforehand that in blennogenous pus the most common form of bacteria is not that of gonococci or curved diplococci of type *g*, *l*, *p* (Fig. 2), but that of common diplococci with round heads, sometimes surrounded by a halo, either within or outside the epithelia or the corpuscles of pus. I have already treated this argument in the other paper upon a case of carcinoma of the bladder, in which the urine showed a considerable number of curved diplococci or gonococci, independently of any blennogenous contagion whatever.

In the Memoir on Whooping-cough, and in the first section of this work, I have touched upon the presence of such curved diplococci or gonococci in the sputa or saliva; as well as upon the singular discovery of true diplococci in a halo, morphologically identical with the so-called pneumococci, in the spermatic fluid recently discharged through illness.

I think that the surprise or incredulity which at first may have been aroused from the present observations will cease, when it is considered that the incentive of all these varied disseminations is always the same, *i.e.*, that the gonococcus as well as the pneumococcus or the bacillus of Koch proceed, in great probability, from small seeds of the same genus; from the parasite that lives normally at the entrance to the digestive and air passages, at the egress of the lachrymal as well as the genito-urinary passages. What wonder, then, if the pneumococcus, towed, so to speak, along the urethra, is found in the spermatic fluid; and the gonococcus, in its turn, is found in sputa and saliva; and that the bacillus of Koch is met with, at times, even in the buccal epithelia of a healthy man ?

For the rest, anyone is in a position to verify the exactness of our observations upon these varied points.* Now from the exhibited facts we must infer that probably the same bacteria or bacilli considered pathogenic (in the affections of the genito-urinary or air passages) are, in reality, only so many disseminations of germs or elements of buccal microbes or balano-prœputialis, from which, morphologically, their varied types do not differ at all; or, at least, that, before admitting the existence of this or that pathogenic bacterium in the above-mentioned passages, it is necessary to demonstrate, with clear and conclusive proofs, that such bacilli, declared pathogenic, are not derivations from the normal preserve. And this objection does hold good, not only in the hypothesis we have set up of the oneness, or duality at most, of the parasitic species of the mouth, but even in the hypothesis now prevailing of their plurality. We contend that the more species there are in the nasal crypts or the cavities of the mouth, the more difficult will it be to exclude their co-operation in generating bacteria reputed pathogenic.

Everything, indeed, leads us to believe that this inexhaustible preserve of normal microbes, placed by nature at the entrance of the digestive and air passages, may have a defensive mission against the intrusion of micro-organisms from without; may, in other words, constitute a true excluding vegetation; a barrier to the effect of preventing foreign germs penetrating and thriving in the adjacent organs.

Nature has imparted to *Leptothrix buccalis* such a power of tenacious resistance to foreign agents that the elements of this fungus upon the human teeth are not destroyed for ages, as it is

* We have lately noticed that our views are confirmed in an observation of Bordoni Uffreduzzi and of Gradenigo, who found "in the pus of the left ear (in a case of ottorhœa) numerous diplococci of biscuit shape, some isolated, others joined in tetrahedrons, partly free and partly contained in cellules, *in the whole similar to gonococci.*" The authors quoted maintain that the various microbes of ottorhœa (in which the lanceolatus diplococcus predominates) originate even from the saliva. *Sull'etiologia dell'otite media. Archivio per le scienze mediche*, Vol. XIV., 1890, pages 276 and 278.

proved from the dental tartar of the Egyptian mummies, in which its filaments have been found intact by Zopf and Miller, by means of dissolving the calcareous salts with acids.*

We would like to make further considerations upon certain points of the present Bacteriological doctrines ; but, in order to keep as far as possible within the clinical area to which our researches are directed, we come to the final conclusion.

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## RECAPITULATION.

In summing up what has been exhibited in the Bacteriological part of the preceding Memoir and the present one, we may conclude as follows :—

I.—Amidst all forms or types of bacteria or bacilli to be found in the sputa, normal or pathological, there exist points of transition, manifesting their polymorphism, and the gradual passage from one type to the other.

II.—Of the types in question, there is not one which cannot be found even in the contents of the mouth, or in the nasal mucus, and the balano-præputialis patina. They do not morphologically differ from each other ; therefore, it is generally maintained that the bacteria and bacilli found in sputa, in normal conditions, are simply secondary disseminations of the buccal or nasal microbes.

III.—But when we deal with bacteria, rightly or wrongly reputed pathogenic—(as, for instance, the pneumococcus or the bacillus of Koch)—it is another matter. We cannot grant their buccal origin, setting up, instead, the hypothesis of their having a specific origin from without.

Nevertheless, even these types cannot be morphologically distinguished from their corresponding types of the contents of the mouth or of the nasal mucus. We should have, at least, to demonstrate, in a positive manner, that the former do not proceed from the latter, although those same microbes are (like the other buccal microbes) thrown by swarms into the air-passages. But that demonstration has not been given ; nay, the only notion upheld

* Zopf, *Die Spaltpilze*, Breslau, 1883, page 80. Miller, *Prehistoric Teeth* (*Independ. Practitioner*, 1884).

from their resisting the decolourising process has, in some instances, been proved wrong.

IV.—Consequently, there only remain, in support of the specificity of such bacteria, the results, more or less controverted, of the methods of culture and inoculation. We shall speak by-and-by about the methods of culture, having already touched on those of inoculation; but leaving, for the present, that question on one side, we wish to deny that the hypothesis of the speciality of bacteria, found in pathological sputa, is at all supported by the general clinical facts, or even by the daily microscopical observation of the sputa.

V.—The congeries of the buccal and nasal microbes, put together, may be summed up to two hundred or three hundred trillions of germs and elements in continual prolification. This vegetation, either through its diffusion—(at least, in many species of domestic animals)—or its constancy and abundance, assumes the character of a true *excluding* vegetation, placed by nature at the ingress of the digestive and air passages, to aid the former in the digestion, and to defend the latter against the micro-organisms of the external world. An identical vegetation adorns the egress of the genito-urinary passages. Thence, it is not surprising if bacteria reputed specific of the air-passages are found even in the products of the genito-urinary passages, and *vice versa;* if, for instance, incapsulated diplococci *(pneumococci)* are found in the urethral mucus or in the spermatic fluid, and curved diplococci (*gonococci* of Neisser, reputed specific forms of blennogenous virus) are found, in their turn, in the sputa or in the middle ear.

VI.—Whatever may be the physiological importance of *Leptothrix buccalis*, it results from our observations that its degree of organisation is far superior to what has been reputed hitherto. *Leptothrix* does not only live as bacterium, bacillus, or filament; but it possesses real organs of reproduction by which it would resemble fungi and *diœcious algæ*, with distinct sexes upon different filaments or individuals. Its fertile filaments are at times engrafted, with two or three roots, upon clods or firm substrata, and end in a fructification. The *ears* constituting these fructifications, as long (in pulmonitic sputum) as 1/6th of a millimetre, are formed of many very minute sporules (so much so that 400 milliards of them hardly would weigh a centigramme); and the small sporules, taking a bright colour with aniline, are disposed in six longitudinal series, making, in some instances, a total of 720 for each *ear*. They are linked together and fastened to the stalk by means of an amorphous substance difficult to be coloured.* However, other filaments, less numerous than these, at times multiple, and lastly, branching off, bear certain productions by *points*, or pseudo inflorescences, formed of spindle-like, snake-like, or comma bacilli (*Spirillum sputigenum*), destined from all appearance, through their lively activity, to the function of conjugation. Finally, there are gemmules in reserve, which (together with the multiplication of the proper sporules) are destined to diffuse the species in the unstable substrata or in the products and in the liquid secretions.

The fructification by *ears* can be reproduced, even in the sputa and in certain small flakes of the urethral mucus.

VII.—Of the six primary species of fungi of the mouth, lately described by Miller, there would, in fact, exist only one—the *Leptothrix buccalis* of Robin (*Leptothrix innominata* of Miller), or, at most, a second one—the Spirillum (*Spirochæte dentium* of Miller). The other four types would represent, if we are not mistaken, only phases or disintegrated particles of the microphyte—viz., *Bacillus buccalis maximus* and *Leptothrix buccalis maxima*, fragments of the stumps that form the inferior layer of vegetation; the *Jodococcus vaginatus* series of special sheaths of bacteria proceeding from certain gemmules of reserve enclosed in the filaments; the *Spirillum sputigenum* (comma bacilli) with our spindle-like and serpentine; appendages detached from the pseudo inflorescences, and probably male organs.

All these particles or articulations cut from the mother plant (except the last—viz., copulative filaments) multiply by themselves, in various ways, according to the condition of the nutrient substratum, in the liquid menstrua or on firm soil.

VIII.—The study of such vegetable forms, in the contents of the mouth as well as in sputa (especially of the fructification by *ears*), requires special rules and care and proper optical means,

* Owing to the connecting of the sporules to the central stalk, by means of peduncles or engrafting threads, visible with a new objective (1/25th in. obective), see a former Note.

without which it would be difficult to verify the facts, even for the most experienced investigators. The productions by *points* are fairly well detected, even with the ordinary objectives; but special care must be taken in collecting and preparing the *patina dentaria.*

We do not know whether the specific oneness of the above forms (by *ears* and by *points*) will be well received by competent observers, as well as all the buccal microbes reunited in a single plant. But, were even two or more of the vegetable species in question, the successive dissemination of their germs and elements in the air-passages would not at all be invalidated.

IX.—The most minute form of cocci, scattered or in a line, found in the contents of the mouth, in the omonimous epithelia, or in the sputa, are, in our opinion, nothing but sporules dropped from the fructifications by *ears*, being first disseminated in the mouth and then thrown into the respiratory passages. The bacilli of Koch (we are speaking of *bead-like* bacilli) might be only these same sporules disposed in series, and thus germinating in the tubercular products and elsewhere. Undoubtedly, the sporules, still attached to the fructifications, or fallen near them, fix strongly the aniline colours; but it remains to be seen whether, in the sputa of consumptives, their resistance to decolourising means is original or simply acquired in the fresh nutrient substratum received from the tubercular lesions. At any rate, that resistance is always relative, and is of doubtful value in the diagnosis, and is sometimes inferior to that of other bacteria, as we have demonstrated before.

The forms of the bacilli of Koch being, on the contrary, in small rods containing granules or vacuoles, with double staining (gentian violet and solution of iodine) behave like the analogous articulations of *Leptothrix*. But upon these and other not less important points, about the clinical study of the tubercular sputa, we shall have to deal on another occasion.

X.—However, if our observations on the morphology and biology of *Leptothrix* are correct; if all, or nearly all, forms of bacteria and bacilli to be found in the sputa, are nothing but *particles* or various organs of a single plant, everyone can see the extent of the actual methods of culture (at least of the bacteria of the air-passages).

And, to be sure, one thing it is to identify or qualify a fungus on the ground of its fructification; another, on the ground of the form of cultures, immersed or creeping, of its single particles, or the modifications brought about by these in the various nutrient substrata. One bacterium or bacillus will fluidify gelatine or elaborate certain principles (even poisons) in a manner totally different from another bacterium or bacillus, without, however, considering the two forms as two organisms or different species, when such forms are for us simply the result of organs or particles of the same plant, destined to attain dissimilar aims, and which may possess the most different qualities.

XI.—Under such conditions, the name of bacterium or bacillus can no longer be considered as synonymous of micro-organism. We may speak of bacteria and bacilli, if by those names we mean particles or articulations detached from the mother plant; and in such a case the generic noun of *microbes* may be even applied. But the word micro-organism applies to the whole microphyte of which bacteria and bacilli are only single scattered particles, and which do not constitute by themselves complete organisms, although they mostly possess the faculty of multiplying on their own account. Such name does not suit at all the single particles, nor, of course, the single bacteria or bacilli.*

I cannot conclude this paper without a short statement.

Perhaps some of my views will appear too bold, but my only intention was to submit them to the judgment of scientific observ-

* In confirmation of our views, we shall quote the opinion of Klein, who lately presented to the Royal Society of London the photographs of a culture of the tubercular bacillus, which appeared with distinct branches like a mycelium fungus. He inferred that *at least a few schizophytes* are really only *forms of development or transitory phases of superior organisms*. The opinion of Dowdeswell is even more explicit. "It is clear" (writes the latter, on the subject of comma bacilli) "that either these microbes are not normal schizophytes, or if so they do not represent an independent group (as it has been observed by the last writers on Bacteriology), but are *simple phases of evolution* of some superior organisms, or perhaps they are only *simple organs of other organisms* (*Lancet*, 1890, Vol. I., *loc. cit.*, p. 1422). Lately, Sheridan Delepine, studying the development of bacteria in its *cultures in interlamellar films* (between the cover-glass and the slide) has come to that conclusion—viz., of the branching off in many bacilli, by means of defined filaments (A New Method, Interlamellar Films, of studying the development of Micro-Organisms, etc., in the *International Journal of Microscopy*, Nov., 1891, p. 343).

ers. I shall only notice that my opposition to certain points of the present Bacteriological doctrines is more apparent than real. I am of opinion that, by pursuing the present method of specifying, in the classification of bacteria, we must more and more multiply their species to such an extent as to hinder the further progress of these studies. On the other hand, should my observations be correct, if the forms of bacteria of the air and genito-urinary passages are reduced to a single species, or if, consequently, even the other very varied types of bacteria (either not at all pathogenic or pathogenic of other parts) might be gradually reduced to defined species of micro-organisms, *according to the natural phases of their full development*; this work upon their arrangement and simplification may aid in the researches of experimental pathology. In other words, we shall be able to recognise the difference between the botanical and the pathogenic entity of bacteria ;' that two or more bacteria, totally different in their own pathogenic action, may proceed from a single micro-organism, and that a bacterium, not pathogenic, may belong, in its turn, to the same micro-organism from which a pathogenic bacterium proceeds.

F. VICENTINI,
Corresponding Member.

*Chieti, June, 1890.*

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For the works of David and Billet, see note at the end of 3rd paragraph.

Plate 3.


F. Vicentini del.

F. Phillips, Sc.

# On Leptothrix Racemosa.

BY FILANDRO VICENTINI, M.D., Chieti, Italy.

THIRD MEMOIR

## On the Cryptogamic Flora of the Mouth.

*Translated by Professor E. Saieghi.*

SUMMARY.

§ 1.—Introductory and Bibliographical Notices. Reference to the previous Memoir. The Works of Pommay, David and Billet.

§ 2.—New observations on the fructification of the normal parasite of the mouth (*Leptothrix racemosa ?*). Various elements and aspects of the grape bunches. Fructification, by temporary spores (*sporids?*), and by persistent spores (teleutospores ?). Varied appearances and forms. Conclusion.

§ I.

### INTRODUCTORY AND BIBLIOGRAPHICAL NOTICES. REFERENCE TO THE PREVIOUS MEMOIR.

WE have often lamented, in many works on Bacteriology, the deficiency of ascertained opinions upon the *Natural History* of micro-organisms. Without an adequate knowledge of the evolutionary cycle of bacteria in nature, from the origin to the varied phases and their complete morphological evolution, we could not even know whether each single bacterium or bacillus, found in a determined material, is an isolated entity, a real and proper living individual, or whether the varied Bacteriological forms are but *membra disjecta*, dissevered and scattered particles, *cellules*, a series of *cellules*, or organs of fungi or superior *algæ*.

We entertained this doubt, in regard to the microbes of the mouth and of the sputa, not through preconceived ideas, but after a series of researches; and we were led to suppose that the common progenitor of all, or nearly all, the bacterial forms of the mucous cavities was probably an alga or fungus which thrives in those parts, the *Leptothrix buccalis* of Robin.

But this first point of the Microphytology, namely, the natural phases of micro-organisms from the scattered or rudimentary forms to those of their complete development, is exactly the part mostly overlooked by bacteriologists, and especially by pathologists; whilst, in our opinion, it is the most important, and, as it were, the basis of all the applications to the pathogenesis of the *infectious diseases* (which is not always the same as that of the *experimental infections*), and without which there would lack the necessary foundation; as the endeavours to investigate the contaminating properties of different isolated bacterial forms, through the inoculations, without ascertaining first, or at least in part, *what are, in reality, the bacteria in themselves, and which, in their natural phases of life*, might be the cause of premature deductions, which a more accurate morphological research would afterwards upset.

Referring to the two Memoirs on the Sputa of Whooping-cough and on the Bacteria of the Mouth and Sputa, we shall simply repeat here our conclusions on the following points:—

(*a*) That the bacteria of the sputa, reputed pathogenic (*e.g.*, the *Pneumococcus* and *Bacillus* of Koch), probably are nothing but so many disseminations, more or less modified, of the same microbes and parasitic elements which normally thrive by hundreds of trillions in the buccal and nasal cavities.

(*b*) That probably the contaminating properties of bacteria in general (such as result from the inoculating experiments) are not to be held as original, but dependent upon simple modifications, occasioned, in single cases, by the nutrient substratum, more or less alterable, on which they fall; or upon the peculiar conditions that their germs meet with in morbid points.

(*c*) That although to a certain extent the usual experiments of culture and inoculation may be able to determine the *pathologica* *entity* of any forms of bacteria, they are yet quite unable to determine the *botanical entity*; in other words, they cannot individualise and qualify their species, as it is possible, generally speaking, to get from the simple disintegration of the same complex micro-organism, different inferior forms or living particles, scattered, eventually endowed with varied contaminating properties, and apt, mostly, to reproduce the original micro-organism; but that, even remaining sterile or simply multiplying in rudimentary condition, does not constitute true distinct species.

(*d*) That in particular, the bacteria and bacilli of sputa do not constitute *complete micro-organisms* or true and proper living individuals; but they are simply *severed* and scattered particles, or inferior forms of only one microphyte.

(*e*) That this microphyte lives normally, not simply in the nose and mouth, but even in the external genitals, and, in given conditions, attains its higher phases of development (analogous to those of fungi or *diœcious* algæ), whose adult forms we have delineated in the preceding plate.

To carry on these investigations, we have made use of a homogeneous immersion objective of 1/18th inch (1·33mm. 1·32 N.A.), by means of which we have been able to detect, in the parasite in question, the existence of those superior phases of life undescribed and unforeseen by other investigators. In fact, we imagined that some productions in the shape of *tufts*, were male inflorescences; whilst, in more numerous productions, we thought we recognised true fructifications or sporifications of this parasite (either fungus or alga) in the shape of so many cumuli of small spores taking a brilliant colour with aniline, placed in line *at the exterior of the fertile filaments*, and disposed in six longitudinal series. This fructification by spores must be distinguished from the gemmules of reserve or production of bacteria *enclosed in the filaments*. Our little spores seemed to be adhering to the stalk through an amorphous, viscid substance, or glair, which hardly becomes colourable.

Although with that objective we could not succeed in seizing the further details of its structure, since then we supposed there were not wanting some real peduncles (sterigmata or engrafting threads) of the single sporules on the central stalk, so slender and transparent that they could not be detected with the optical instruments employed at that time. Therefore, having left the matter undecided, we proposed the name of *ears* for those fructifications.

The preceding Memoir was in the press when we kindly received from Messrs. Bézu, Hausser, and Co., a new 1/25th inch homogeneous immersion objective; this objective revealed at once the existence of the predicted peduncles or engrafting threads, so that we were able to give a previous hint of it at the time, proposing then to change the first name of *ears* into that of *bunches* (as such are really the fructifications of *Leptothrix buccalis*). We then promised to deal more specially with the subject in a new communication.

We will, therefore, relate the most salient facts connected with the fructifications and other superior phases of our parasite, and delineate the more important types of it, making use only of two stains, gentian violet and an acidulated solution of iodine, discarding all other stains. It would be inopportune now to go more deeply into this morphological study, without having first confirmed the essential points which more exactly concern those adult phases investigated and described by us for the first time.

The objective adopted for these new researches, and of which we gave a hint in the note above referred to, is not included in the last catalogue (1891) of its constructors. It is a very solid objective of N.A. 1·30, the immersion fluid being vaseline oil. It has an equivalent focal distance of 1 mm., provided with a correction collar, and possessing a proper or initial magnifying power of 180 diameters, which, with the various Huyghenian eye-pieces, magnifies from 820 to 3,100 diameters. The working distance is relatively considerable. The images are perfect over the whole field, even with the No. 6 ocular, so that with it may be detected from forty to fifty red corpuscles of the blood (each one of them about 26 or 27 m.m. in diameter) in the same visual field and plane; but, beside its great magnifying power, it is to be commended for its resolving power, which is not inferior to that of apochromatics. The instrument we have made use of is the No. VII. inclined stand of the same makers, with a stage one decimetre square, revolving with the superior part round the optical axis. The stand has an Abbe condenser of

1·40 N.A. (with centring and focussing motion), and is provided with an iris diaphragm and dark-ground illumination.

Before proceeding to demonstrate our new observations, it is necessary to complete the Bibliographical notices briefly described in the previous Memoir. We made a point of perusing all the publications upon this subject that we could obtain, in order to ascertain whether others had anticipated us, or met with analogous results, in regard to the superior phases of *Leptothrix*. We will, however, only touch upon three works, published from 1890 to 1891; the more so as the same works (although silent on the above-mentioned phases) offer us, on the other hand, points of agreement with our various observations and views on the inferior phases of microbes in general, and in particular of those of the mouth and sputa.

### Article by Pommay. *

One of the most important points of the modern bacteriological doctrines is to know whether, in the experiments of the laboratory, the virulence of certain materials or cultures, containing bacteria, is to be held as original and part of the microbes in question, or simply an accidental acquisition. Another point, not less important, is also to know whether, in certain cases, the virulence as such is not to be assigned to the material in which they thrive, or to their products of secretion, rather than to the microbes themselves.

The present work by Pommay is not the result of original researches, but rather a retrospective review of many previous observations gathered from different authors. In the first part the author largely deals with certain conditions, which, although extraneous to bacteria, are nevertheless to be held as the true agents of the virulence. It has been proved that the inoculation of substances secreted by the bacteria often gives rise to nearly the same morbific phenomena attributed to the relative microbe as such. Pasteur, by inoculating the sterilised liquid cultures of chicken cholera, has reproduced the principal symptoms of that disease. As much can be said of the *Bacillus pyocyaneus* and the

* H. Pommay, *De l'origine et des conditions de la virulence dans les maladies infectieuses* (*Annales de Micrographie*, III., pp. 220—74.)

cholera bacillus (Gamaleïa) of the staphylococcus (Christmas), and the *Bacillus anthracis* (Hankin), etc. To these examples more recent ones might be added, but it is enough to point out the case of the tetanus-virus. After the experiments of Gaillard and Vincent, Kitasato in a recent article affirms that the cultures of tetanus bacilli, filtered after Chamberland's Method, produce *absolutely the same tetanizing action* as the cultures still containing bacilli.*

"Virulence," concludes Pommay, "is not at all a necessary function of microbes; the secretion is an essential act of life of the cellule . . . . but the nature of the secretion depends only upon the total of the specific conditions of each micro-organism, and follows up its variations in such a manner as to be more or less influenced by the various changes of the external agents" (pp. 221—22).

At this point Pommay touches upon some particulars concerning the influence of the external agents upon the virulence, the dissolving power and other properties of bacteria, which we do not consider. "Virulence," he adds, "namely, the secretions of poisonous matters, may be increased, reduced, and even suppressed by natural agents, or by artificial means. We can, therefore, affirm, that virulence is nothing but a *contingent function* of the micro-organisms, that it depends chiefly upon the external agents, and the microbes do not evolve from themselves or their own substance, but from substances on which they thrive and multiply. The soils or media of cultures may, in respect to this, be divided into indifferent, positive, and negative soils. The indifferent soils have no action whatever upon the functions of bacteria; if these are virulent they secrete their virus, and if not virulent, they do not become so. The positive soils do not only supply the materials of virulence, but they modify the microbes so that the virulence, occasionally lost by them in the negative soils, is revived. In the negative soils the virulent microbes lose the aptitude of secreting poisonous matters, and it is only through passing into a positive soil, that they will become virulent again.

* *Zeitschrift für Hygiene*, X., page 267. We, however, begin to suspect that an eventual passage of very minute bacterial particles through the porous porcelain takes place. V. Freudenreich, *De la perméabilité des filtres Chamberland à l'égard des bactéries.* (*Annales de Micrographie*, IV., p. 559) F.V.

In conclusion, the virulence may, in a certain manner, virtually exist apart from the microbes; these embody it, and set it to work; *it is an acquired and transitory function of the pathogenic micro-organisms, and not an essential function inherent to their existence, and it is subject to different external influences*" (pp. 227—28).

The author, afterwards, dwells upon the changes undergone by bacteria in the air, water, and soil. He infers that the pathogenic bacteria are found in a very small number round about man, but that it is necessary to look for them in man himself. He here gives instances of pathogenic bacteria found in the mouth, nose, and in normal sputa, in the same way already described by us in our memoirs.

In the second part, Pommay begins by observing that simple saprophytes may evolve themselves in the sense of virulence, adapting themselves to new conditions of existence, and that infectious diseases, whilst partly proceeding from pathogenic germs evolved in individuals already invaded, can, on the other hand, proceed from germs originally inoffensive, having become virulent outside the *economy*, through the influence of the nutrient substratum. The adaptability of micro-organisms is so unlimited, and the time required for their metamorphoses so short, that the passage from one phase to another seems to us instantaneous; consequently we incline to make *two different individuals* of forms which, in reality, *proceed the one from the other.*

It is quite probable that the bacteria considered pathogenic may not be, and have not been always, such, but may have lived previously, and may still live in process of time, as simple saprophytes. The author thinks that the latest researches strengthen that probability to a degree of certainty.

The bacillus of Lustgarten, considered to be the bacillus of syphilis, is so like the bacillus of smegma as to be held for the same normal bacillus, *modified in its action,* after having long lodged in the materials of secretion heaped up and fermented upon the genitals. A normal bacterium of the urethra is probably the progenitor of the *Gonococcus* of Neisser (see our preceding notes and memoirs). The bacillus of diphtheria exists in the condition of a simple saprophyte in the mouths of healthy children (Roux and Yersin). Likewise the bacillus of Eberth, the specific germ of *ileo-typhus*, is considered to originate from *Bacterium coli commune* (Rodet and Roux, of Lyons).

"The bacillus of Lustgarten, the *Gonococcus* of Neisser, the bacillus of diphtheria, and the bacillus of Eberth, would, consequently, be found (concludes the author) in us and upon us, in the state of saprophytes, thriving inoffensively upon those same parts that they attack at the moment of the virulence. From such similarity of seats, we think of the relation existing between the saprophyte and pathogenic microbes, or rather of their transformation *sur place*" (p. 262).

This idea is strengthened by many historical arguments.

Many infectious diseases which are of more frequent occurrence nowadays, did not formerly exist; some, which were once common, have now disappeared, or tend to do so; others show alternatives of activity and repose. A secluded life, for instance, has favoured, in our times, the appearance of typhoidal diseases and the spreading of tuberculosis.

Yellow fever is comparatively recent, having appeared since the shores of the Gulf of Mexico and the Great Antilles have become covered with populous centres; and, unless we presume a spontaneous generation of new species of micro-organisms, we are bound to admit that the specific microbes of yellow fever (if to this disorder a parasitical origin is to be assigned) are only a transformation of other common pre-existing microbes, consequent upon the contamination of the soil, the overcrowding population with the co-efficients of climate, misery, etc.

Leprosy (formerly so common) is now confined, in European countries, to very few localities, and even where it appears does not make many victims, having nearly lost its virulence. We must, therefore, admit an attenuation, and also presume a successive disappearance of the virulent action of its bacillus, etc.

Other infectious diseases alternately appeared and disappeared. A singular instance is found in the sweating disease which appeared for the first time in 1486, and four times up to 1530, when it finally disappeared. We cannot imagine that the germs of the first epidemic had been preserved until the appearance of the second, and so forth; but we must argue that the germs were always present, at every epoch, only becoming virulent at intervals.

Analogous cases are exhibited by a similar disorder in Picardy. Exanthematous typhus broke out in Algiers in 1867, without being imported.

We must, therefore, according to Pommay, distinguish the period of formation of an infectious disease from its period of diffusion. In the first period we have the evolution of common bacteria in the sense of virulence; in the second, the multiplication of the germs themselves, owing firstly to their transmission from one to the other affected organism, and secondly to their extraordinary multiplication in excrements and secretions, or in infected corpses.

Setting aside the considerations concerning hygiene, into which the author then enters, we will simply give his final conclusions, which so nearly agree with our own views :—

I.—" The virulence is not a primitive, original, and necessary condition of pathogenic microbes; it is an acquired, contingent condition, which proceeds from the evolution of certain microbes, in certain conditions and soils.

II.—" Of Culture media, some increase or create the virulence; others attenuate or extinguish it; others are indifferent—that is, neither modify nor produce it.

III.—" The pathogenic germs proceed either from morbid points in which the virulence is acquired or maintained, or from individuals affected with the disorder due to such germs. The prophylaxis, to be called certain and complete, must, on one side, ascertain and suppress the conditions producing the virulence; and, on the other, suppress or destroy the germs proceeding from the diseased bodies, or impede the propagation of them.

IV.—" Water being only an intermediate between the organism and the pathogenic germs, the prophylaxis based upon the purification of water is uncertain and incomplete.

V.—" The pathogenic germs, taking up their virulence in the soil or in the organism, must be attacked in those two fields; the purification of the waters coming next.

### The Work of David.*

David, late director of the Odontological School of Paris, is already known by other works. The one to which we now refer, may be properly called a work of compilation, following in the footsteps of Miller, and, although very valuable of its kind, helps in a measure to complete Miller's work.

In the first chapter he gives a historical summary upon the microbes of the mouth from Leeuwenhoek to the present time, in which he especially mentions the last works of Pasteur, Netter, Vignal, Miller, Blake, Galippe, and Biondi, quoted by Miller in various places. The observations of Netter and Vignal upon the presence of pathogenic microbes in the mouth of healthy subjects are particularly worthy of notice. Vignal found in his own mouth two staphylococci, and such researches agree with those of Sternberg on the presence of the pneumococcus and the tubercular bacillus of Koch in healthy subjects (Comp. with our work on the *Sputa of Pertussis).*

Omitting the brief notices of Microphytology and Biological Chemistry, hardly touched upon by the author, we come to the second chapter (description of the common microbes), where are, in the first place, exhibited the forms of *Bacillus subtilis*, *Bacterium termo*, *Bacillus amylobacter* (or butyric ferment), the bacillus of potatoes and of the *Vibrio rugula.*

In the number of common bacteria, the author even includes *Spirilla* and *Spirochœte*, although he himself, further on, speaks of their infectious action upon the tissues and circumambient glands, already recognised by Verneuil and by Clado.

We have elsewhere treated of the genesis of *Spirilla* and *Spirochœte*, though omitting an important question of their biology, which we now find hinted by David. That question concerns the possible affinity of the *Spirochœte* with the brilliant granules, their staining with aniline, and vibrating with a dancing motion, which are lodged in swarms within the salivary corpuscles (see Fig 1, *h*). Rud and Arndt suppose that such dancing granules are nothing but germs of Spirochœte, temporarily sheltered

* Th. David, *Les Microbes de la bouche, précedé d'une lettre préface de M. L. Pasteur*, Paris, 1890, 8vo, pp. xvi.—302, with 113 figures intercalated, in colours and in black.

in the salivary corpuscles, from whence, being afterwards set free, they would constitute germinating spores for producing new Spirochœte. But the affinity of those dancing granules with the so-called microzymes, and with bacteria in general, had been already foreseen by other observers, as was remarked by Beale.* At present that affinity would be also connected with the fact, not less important, of the normal nesting of bacteria within the white corpuscles of the blood, ascertained by Baumgarten, which is diametrically opposed to the hypothesis of phagocytes.†

David proceeds to describe the *Bacillus tremulus* of Rappin, the characters of which we have studied elsewhere; in our opinion, it is one of the male elements of *Leptothrix buccalis*.

We are surprised that David admits the presence of *Leptothrix* only in the Carnivora; whilst a nearly analogous parasite has been recognised in most domestic herbivora and in swine by Miller and other investigators. But the author is right in doubting the separate entity of *B. buccalis maximus* and *Leptothrix buccalis maxima* of Miller; and this wholly agrees with our views.

With regard to the pointed granules contained within the filaments of *Leptothrix*, the author entertains the same opinion as Robin, namely, that they might be spores. We, on the contrary, incline to hold them as *gemmules of reserve*, destined, in the severed or truncated filaments, to become ellipsoidal bacteria, or even dumb-bell bacteria, according to their respective position in the envelope; whilst the real spores should be found on the top and *outside* of the fertile filaments.

The observation of Rappin (p. 67 of this work) is rather important respecting certain filaments of *Leptothrix* found near their engrafting point, *in continuity with some little chains of bacteria*. David only sees in it an optical effect, due to the superposition of rosaries of *Streptococci* with slender filaments of *Leptothrix*; but, in our last memoir, we have already described and delineated an identical fact (preceding memoir, Fig. 9, *d*), the same unquestion-

* Beale, *The Microscope in Medicine*, London, 1878, p. 272.

† Baumgarten, *Zur Kritik der Metschnikoff'schen Phagocytentheorie (Zeitschr. f. Klin. Med.* Bd. XV., 1888.) *Ueber das Experimentum Crucis der Phagocytenlehre* (Zeigler's *Beiträge z. path. Anat. u. allg. Path.* Bd. VII., 1890, p. 1.)

able continuity of filament with the small chain, which will lead us to the genetic identity of the two forms; identity at all events in harmony with the other forms or morphological phases of *Leptothrix*.

David subsequently deals with the fungi of Biondi and of Vignal already mentioned in our last memoir. At the close of the chapter he hints at the digestive action of the buccal microbes, and then at their fermentative action upon lactic acid, mannite, dextrine, butyric acid, and other substances (see quoted Memoir).

The third chapter (*Pathogenic microbes found in saliva*) deals, in the first place, with the forms of the pneumococci of Fraenkel and Friedlaender clearly detected in the mouth even of healthy subjects. On this point we refer to our two previous memoirs, and wish only to state that the casualty or accidentality of the pneumococcus of Friedlaender was recently demonstrated by Gamaleïa*, and we may, some day, obtain the same results with the pneumococcus of Fraenkel. Probably such diplococci, incapsulated or bare, are but a condition or particular phase of certain particles or articulations of the same normal parasite of the mouth; a common condition of other bacterial species, which Billet calls the *zoöglœic state*, as we shall see later on.

This chapter ends with the description of the so-called microbes of suppuration (*Streptococcus pyogenus*, *Staphylococcus pyogenus*, white and golden).

In the fourth chapter (Pathogenic microbes found in buccal and dental affections), the author deals, firstly, with the tubercular bacillus. That the bacillus of Koch was also liable to decolourisation with nitric acid (at $\frac{1}{3}$) and alcohol, although in a degree (generally speaking) less than other bacteria and bacilli, it was an acknowledged fact even by Jacksch and Hunter Mackenzie, as we have said before. The opinion of David is virtually nearer to that of the authors mentioned. In the first place, the solution of nitric acid, recommended by him in following the method of Ehrlich (p. 135) for decolourising the preparations made by dry processes, is found notably weakened; it is no longer a $\frac{1}{3}$ solution, but rather a $\frac{1}{4}$ or $\frac{1}{5}$. Secondly, the immersion in the nitric bath

* Gamaleïa, *Annales de l'Institut Pasteur*, 1888.

has been gradually shortened. For Jacksch it was only a question of reducing the violet to green; for Hunter Mackenzie the length of thirty seconds could not be exceeded; and now David does not allow the nitric bath (although doubly diluted) to last more *than two or three seconds*. Dr. Kauffman, of Cairo, has lately found that the bacilli of Koch, coloured with carbolic fuchsine, retain the tint only five minutes, if washed in hot or boiling water. He uses it instead of nitric acid and alcohol, but only for two minutes.*

As we see, the opinion that the bacillus of Koch had a very special retentiveness for aniline has been gradually losing ground. At any rate, this is not the only bacillus which fixes tenaciously the aniline, and we exhibited specimens, especially of diplococci, in long envelopes. The bacillus of smegma and of leprosy have the same property. Gottstein and Bienstock thought that the resistance to decolourisation in those bacilli might depend upon a protecting envelope of a fatty substance; and in fact, Grigorjew cultivating other bacilli (*Bacillus anthracis*, *B. subtilis*, *Clostridium butyricum*, *Bacterium termo*, and the two Staphylococci), in fatty *media*, affirms to having found them equally resisting.†

Of the other microbes grouped by the author under this same heading, we shall only mention the *Micrococcus tetragenous*, *Oidium albicans*, *Actinomyces*, and then the fungi of Miller (including the *Jodococcus vaginatus* and the *Spirillum sputigenum*), and the already mentioned fungi of Biondi. At page 206, a *Leptothrix pusilla* is dealt with, described by Klebs as the *ceiling* of the dental tartar, but it is discarded by the David.

The fifth and last chapter (Practical and therapeutic deductions) does not concern bacteriology. The appendix on *The Microbes of Influenza*, preceding the last studies of Pfeiffer, arrives at unsatisfactory results.

* *Modern Medicine and Bacteriological World*, May, 1893, p. 126.

† Gottstein, *Deutsch med. Wochenschr.*, 1886. Bienstock, *Fortschr. d. Med.*, IV., 1886. Grigorjew, *Ruskajä Medizina*, 1886.

## The Work of Billet.*

Billett, Surgeon-majör in the French army, had already published other works upon the *Sporulation of Cladothrix dichotoma*, upon *Bacterium ureæ* (1885), upon *B. laminariæ* and *B. Balbiani* (1888). The present work, for which the Institute of France awarded the Montaigne prize for 1890 upon the report of Bornet, has been published in full in the *Bulletin Scientifique de la France et de la Belgique*, by Prof. Giard. It is divided into an introduction and four sections (one on each of the described species), besides a rich bibliographical Appendix, comprising 662 works, monographs, or groups of articles, upon that or kindred subjects.

Examining the simple phases, which we call inferior, of the micro-organisms, Billet goes to demonstrate a thesis analogous to ours on the *Leptothrix buccalis*, and to establish a similar genetic identity amongst the varied bacterial and bacillary forms proceeding from four other species of micro-organisms (*Cladothrix dichotoma*, *Bacterium Balbiani*, *B. osteophilum*, and *Leptothrix parasitica*, Kützing, a parasite of the superior sea-weeds), and he comes to the conclusion that such micro-organisms may even be algæ analagous to *Cyanophyceæ*, and that, like those, they present themselves either under the form of a fibrous sprout, or of isolated and motile elements, as the *hormogonia of the Nostocaceæ*. The *Cladothrix dichotoma* resembles in the sporulation (which we call gemmulation) the *Nostoc ellipsosporium*, described by Bornet. The *zoöglœic state* reminds us, in its turn, of analogous forms, described as the *Chroöcoccaceæ* (a group of the same Cyanophyceæ). The absence of the chlorophyll is not sufficient to relegate such micro-organisms to the family of fungi or schizomycetes, after that Van Tieghem and Engelmann have demonstrated the pigment of certain Bacteriaceæ of a green and purple colour to be a chromophyll (analogous to chlorophyll), by means of which such plants decompose, on one side, the carbonic acid under the action of light, and on the other, produce the synthesis of the hydrates of carbon.

In the introduction the author recapitulates the history of the

* A. Billet, *Contribution à l'étude de la Morphologie et du développement des Bactériacées*, Paris, 1890, 8vo, pp. 288, with 19 figures and 9 plates, some coloured.

*Bacteriaceæ* : a group of organisms (he says) of which the study has much interested biologists ; still, there are members of the vegetable kingdom whose history is so scantily cleared up. Under the generic name of *Bacteriaceæ* adopted by Van Tieghem, the author designates the whole of the microbic forms commonly called *Schizomycetes* or *Schizophytes* ; the morphological study of which, inaugurated by the first investigators (from Leeuwenhoek to Hoffmann) has been afterwards abruptly abandoned in order to follow, almost exclusively, the phenomena connected with their biological action on fermentations, putrefactions and infectious diseases, as investigated by Pasteur and Koch. The small number of morphologists who continued the morphological research, soon divided into two fields : one maintaining with Cohn the immobility of bacterial forms ; the other sustaining with Ray Lankester, Cienkowski, and Zopf the theory of pleomorphism or passage from one form to another, provided that this passage be not taken, nowadays, as a *transmutation from one species to another*, but as a series of *various phases common to appurtenances of more complex species.*

The first investigators examining the bacterial forms in liquid substrata, became acquainted only with their isolated condition, and they created the species of *Cocci*, *Bacteria*, *Bacilli*, *Vibrios*, *Spirilla*, and *Spirochæte.* The first modification of these absolute ideas is found in Robin, who, in the *Leptothrix buccalis*, shows the affinity between the *Bacilli* and the *uncoloured filamentous algæ*, purposely called *Leptothrix* by Kützing. And, in fact, we have to admit that even other species (viz., the genera *Beggiatoa*, *Cladothrix*, *Streptothrix* and *Crenothrix*) assume, besides the *dissociated forms*, the *filamentous forms*. Cienkowski (1877) demonstrated that the forms of bacteria were transformed into cocci, and that the cocci in their turn assumed the form of *Leptothrix* ; he called the attention of the microphytologists to the *zöoglœic formations*, mentioned by Cohn in 1853. He verified this zöoglœic phase in the genera *Cladothrix*, *Crenothrix*, and *Leptothrix* together with the preceding phases. In 1881 Zopf extended the like views to the genus *Beggiatoa* ; he showed that the filamentous forms can be dissociated in Bacilli and Bacteria or assume curved, vibrionic, spirillic or spirochætic forms ; he explained the genetic connection and demonstrated that certain *zoöglœic formations* held as bacterial independent forms, constitute in reality but one proper phase of the same filamentous species.

The views of these authors, generally adopted for some of the highest types *(Cladothrix, Crenothrix* and *Beggiatoa)* were not extended to most of the bacterial species. The three mentioned species formed as a distinct group, whilst for the others we entertained the primitive views of Cohn. But later on new exceptions were introduced, which have now so far multiplied as to become the rule. The author quotes the three species of *Proteus* of Hauser, the *Helicobacterium* of Escherich, the *Bacterium carotarum* of A. Koch, the *Bacillus brassicæ* of Pommer, the *B. cæruleus* of Smith, the *B. pyocyaneus* of Flügge, the *Spyrobacillus Cienkowskii*, etc. Only for the ancient genus *Micrococcus* an absolute separation is still maintained, but (adds the author) the *Pneumococcus* itself shows to Friedlaender, Pipping and E. Klein, gradations to the rectilineal forms. The same may be said of *Micrococcus ureæ* and of *Micrococcus prodigiosus.*

Billet should have mentioned the last work by Miller *(Die Mikroorganismen der Mundhöhle*), where his return to the preceding views appears evident, but it would appear from the bibliographical index that it was not then known to him.

"The theory of the genetic relations of the different bacterial forms" (continues Billet) "is not simply based upon exceptional cases and speculative views. It has been, and is yet sustained by workers whose methods cannot be suspected, nor their talent for observation and interpretation questioned. To us the morphological question of the Bacteriaceæ is even wider. The point is not only to know whether such and such species present a succession of forms proceeding one from the other, but, above all, to know whether the Bacteriaceæ have an evolutionary cycle in morphological characters particular and constant to each species, the general development of which depends upon a law common to the greatest part of them. In fact, from data in our possession, we can easily perceive that most of them pass through various phases during their evolution, according to their conditions of life. Thus, in a first phase, the bacterial elements are found associated in a *filamentous graft,* more or less long; in a second phase, these elements become free and motile ; in a third, they group themselves in gelatinous masses more or less considerable, and which, to some of them, seem characteristic. Lastly, in a fourth and last phase, it may happen that the preceding filaments intertwine one with another in coiled masses at times very voluminous. We describe these four phases more particularly as the *filamentous*, *dissociated*, *interlacing* (*enchevêtré*), and *zoögloeic* states."—pp. 21–22.

We cannot follow the author in the special chapters devoted to each of the four species studied by him. We shall only show some points which may, in our opinion, clear up certain questions connected with the microbes of the mouth and sputa.

And firstly, the dissociated and zoöglœic states, considered as simple phases of filamentous bacteria render it possible that diplococci or pneumococci may have been derived from the normal leptothrical filaments themselves, as we have noticed elsewhere.

In fact, if we look at the relative figures of Billet, reproduced by us, we may take them, at a first glance, for true pneumococci rather than for simple disjunctions or simple zoöglœic groups of the algæ studied by the author. In Figure 17, *a* represents a fragment of six articulations of *Cladothrix dichotoma* in disjunction, magnified to 320 diameters, which, except in its greater size, does not differ from the pneumococci. In *b* are noticed analogous articulations magnified to 1,600 diameters, in a more advanced stage of disjunction. In *c*, *d* are seen two specimens of the *Bacterium osteophilum* in its passage to the zoöglœic state, magnified to 600 diameters. In *e* is drawn a fragment of four articulations of the same Bacterium in that particular zoöglœic form called by Billet the *Scorpioidal state*. In *f* is reproduced a small group of the zoöglœic state of *Leptothrix parasitica* Kützing, magnified to 745 diameters, and their perfect resemblance to the sheathed diplococci, reputed specific, is not less evident. Other similar specimens are reproduced in Figure 18, *a* (stained with solution of iodine and afterwards with methyl violet or fuchsine, magnified to 600 diameters), where are detected rectilinear forms of *Cladothrix dichotoma*, analogous to the sheathed diplococci drawn by us in the preceding plates (Fig. 2, *l*, Fig. 4, *g*), in their stage of passing to the zoöglœic state. In *b* (stained first with vesuvine, then with methyl violet and solution of iodine) is seen the beginning of the zoöglœic state of *Bacterium osteophilum*, magnified to 600 diameters ; in *c* the beginning of the *scorpioidal state*; and in *d* a larger representation of the first state (1,600 diameters). In *e* (stained the same as *a*) is seen a zoöglœic group of *Leptothrix parasitica*, magnified to 320 diameters. All those figures clearly show their analogy with the pneumococci.

The other point we think worth considering is the formation (in *Cladothrix dichotoma*) of the so-called *zoöglœa terminalis* predicted by Cienkowski and demonstrated by Zopf, under the name of *zoöglœa ramigera*. This form is particularly produced in the cultural liquids containing animal substances in putrefaction. A specimen of *arborescent zoöglœa* of *Cladothrix* is seen in Fig. 19 with 21 branches upon a single peduncle (coloured like Fig. 18, *a*, magnified only to 120 diameters). In *a*, *b* (Fig. 20, similarly coloured, magnified to 600 diameters) are drawn the ends of two clavated branches, in order to show their contents, which are formed of bacteria of various sizes and shapes ; these are better observed in *c* of the same figure, magnified to 1,050 diameters.

Now such forms simply heaped up must be distinguished from the fructifications by *ears* or *clusters*, already described in *Leptothrix buccalis*, for their arborescent disposition (in the zoöglœa), for the irregularity of their outlines, their much larger size, the mixture of heteromorphic bacteria in the same mass, and finally for the want of the internal stalk which is very apparent in the *ears* ; and that, apart from the last fact verified by us of the peduncles or engrafting filaments of the little sporules of *Leptothrix* on the central stalk. Of such *external sporulation* upon certain fertile filaments and of the production of tufts (probably male organs) upon other filaments, not a word or even a hint is to be found in either Miller, David, or Billet.

Being obliged to pass over the brilliant researches of the author upon each of his four species, we will here give a partial summary of his conclusions.

I.—The various states of the *Bacteriaceæ* correspond to a special grouping of their elements in relation to the conditions of the nutrient substratum (composition, temperature, pressure, aeration, &c.) Therefore nothing is fixed in the succession of those phases ; one state can rapidly evolve towards another, or fail, or remain unchanged in the so-called *pure culture*, viz., in a medium of known and unvariable composition under the same conditions of temperature, pressure, and aeration.

II.—The *filamentous state* is pre-eminently the vegetative state. Here the bacterial elements are disposed in small chains or articulated filaments (by division of a primitive element in a single direction) sometimes enveloped in a real filamentous sheath, sometimes simply joined by bands of intersticial substance or glair (of the same nature as the sheath) secreted from the bacterial element itself, and which, when it is more abundant, involves the groups of the *zoöglœic state.*

III.—The bacterial elements of the filaments occupy the internal part under the three rectilineal forms in *Leptothrix*, *Bacillus* and *Bacterium ;* and in the three curved forms, *Vibrio*, *Spirillum* and *Spirochœte ;* forms differing between themselves but all proceeding from the primitive rectilineal element. It is no question of the *pleomorphism* of the old hypothesis nor of change from one species to another, but simply of change of state.

We pass over what the author positively states about the form in *Micrococcus*. He only found it once in *Bacterium Balbiani* and he believes that it proceeds from the same elements *in Bacterium*, reduced to a minimum of length through a change of the culture medium. In fact, by restoring the medium to its previous conditions the primitive form was resumed. In *Cladothrix* and in *Bacterium osteophilum*, on the contrary, the roundish elements proceeded from a retraction of the protoplasm *evolving itself towards the spore.* On our own part it is enough to observe that in *Leptothrix buccalis* the roundish forms are very common, and (in all probability) proceeding from true spores. The four species studied by Billet (for reasons which we shall not investigate), did not exhibit a true sporulation (on the exterior of the filaments) which, we think, would explain the total or partial absence of the micrococci.

Returning to the filamentous state, the condition more apt to develop it properly is, according to the author, on one side the fluidity of the nutrient substratum, and on the other the nearness to the surface. It is essentially an aerobic state. Another character which distinguishes it is that it generally has non-motile filaments.

IV.—" The *dissociated state* is characterised by setting free the constituent elements of the filamentous state. These elements continue to disintegrate, but instead of uniting into series or chains they separate and live isolated. At times, however, they remain united in couples or in small chains, but dissimilar from the preceding elements, they are essentially motile. Consequently in the dissevered state all those forms will be found which have been already described in the filamentous state. Physiologically, the dissevered state has a very important office to fulfil, which is chiefly a phase of dissemination. Thanks to the mobility and the active segmentation of its elements, it can in a short time spread over a very extensive culture medium. Thus, it so happens, that under these very forms of isolated, dissevered or motile elements the Bacteriaceæ are more frequently met with, and are better known.

V.—" The *interlacing state* constitutes a third phase of the developing cycle of the *Bacteriaceæ*. It shows itself under the form of filaments interlacing into one another like so many threads in a piece of knitting. Physiologically, this is a transitory state between the dissevered and filamentous states, or between one of those two states and the zoögloeic state.

VI.—" The *zoöglœic state* is the last phase of the evolutionary cycle. The elements of the preceding states group themselves according to certain dispositions which, at a first glance, do not exhibit any apparent order, but in reality they follow up a definite order, which in the *Bacteriaceæ* we have considered varies for each species. The chief phenomenon is the secretion round each element of a viscid substance or glair, morphologically and physiologically identical with the sheath involving the filaments. By the same fact of the increase of this gelatinous envelope, which surrounds them like a capsule, the elements become motionless."

The zoöglœic state afterwards behaves differently through the various stages, which we will briefly notice. In the first stage (corresponding to the form already described) each element is enveloped in a proper gelatinous capsule, and the morphological aspect of the elements entirely corresponds to that which characterises the genus *Hyalococcus*, created by Schröter for the *Pneumococcus* of Friedlaender. In a second stage of the same zoöglœic state, we have the forms of *Merismopedia* or *Tetrads*, and in a third state the form of the *Sarcina*. To this form we think should be related the straight and curved diplococci of types *g*, *l*, *p* (Fig. 2), from which the so-called *Gonoccocci* of Neisser would then be derived. Sometimes we have also the forms of *Ascococcus* or *Zoöglœa terminalis*, of which we have made a mention before.

Physiologically, the author believes that the zoöglœic state constitutes for a large number of the *Bacteriaceæ*, a *protecting phase* of the relative elements against the external agents, or of preparation for more favourable conditions in which to be able to abandon the gelatinous envelope in order to thrive again in the filamentous or dissevered state. As regards the formation of *endogenous spores*, we could not, without some reserve, adopt the opinion of Billet, having already elsewhere expressed the idea that such bacterial forms enclosed in the filaments, are only (at least in our parasite) a development of the gemmules of reserve. Such gemmules are not, on the other hand, to be taken for the *granulations*, considered by him as *products of reduction*, due to a *degenerative phase* of the respective articulations.

From these remarks the author concludes that in order to determine the bacterial species, it is no longer sufficient to describe their isolated forms in the *single soils* or media; but it is necessary to investigate whether any forms remain always constant, even in different media. Then many forms held formerly as distinct species, will simply appear as accidental of the same varieties.

The author lastly asks what are the probable affinities of the *Bacteriaceæ* with other vegetal groups, and, having discarded (as we saw before) the analogy with fungi, he brings them nearer to the cyanophyceous algæ (*Nostocaceæ*) according to the original opinion of Cohn and the ideas of Van Tieghem and Bornet. And for the following reasons:—(1) Their filamentous development in one single direction, like the trichomes of the filamentous *Nostochineæ*; (2) their motion, analogous to that of the *Oscillariaceæ*; (3) their dissevered state, similar to the *Hormogones*; (4) their zoöglœic state, analogous to the tabular sprouts of *Merismopedia* and *Glœocapsa*; (5) their endogenous germination of the spores (in the *Cladothrix*), analogous to that of certain nostocs.

"Finally" (he adds), "we cannot do better than repeat the words of Dallinger:—*The bacteria are without much question a botanical group, and to be thoroughly understood must be studied as such.* In order to know them and to be able to catologue their species, as we have done for the other vegetal families, we have, then, to investigate closely their phases; and when we shall be better acquainted with the true being of their species, we shall be able to study more accurately their true office, their functions, and physiological properties."—(pp. 208–18.)

When, in October, 1890, we presented the preceding Memoir, we were not acquainted with the above-mentioned works. Seeing now that our views are, at least in part, shared by these and other competent investigators, we hope the opposition to the course we have followed in these studies will vanish or be minimised, and that the present remarks (although incomplete) will induce others to initiate further and more accurate researches.

We pass now to describe our last investigations upon the fructifications *by clusters* and the other superior forms of the normal parasite of the mouth.

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§ II.

## NEW RESEARCHES ON THE FRUCTIFICATION OF THE NORMAL PARASITE OF THE MOUTH

*(LEPTOTHRIX RACEMOSA?)*

I TRUST it will not appear too pretentious on my part if I propose for such an isolated form—or, better to say, for the tiny plant which thrives (and appears to germinate and fructify)—on our teeth, taken as a whole, the name of *Leptothrix racemosa*, instead of that of *Leptothrix buccalis*; and this solely on purpose to qualify it better, if it be true that the cryptogamic species must take their name and character from the fructification (if any) rather from the inferior or rudimentary appearances of their single particles, incidentally incomplete, scattered, or isolated.

In describing the various aspects of the fructification in question, we shall endeavour to group them in such a way that, according to our view, there may be a better connecting of the varieties; but, in doing so, we do not wish to prevent a more matured judgment.

### Various Elements and Aspects of the Bunches.

To understand the various aspects which the ears or bunches of *Leptothrix racemosa* present, we must value the different constitutive elements of those clusters, which, according to our observations, we consider to be four, in their natural order of development.

The principal element, from which the other three proceed and upon which they are formed, is the fertile filament, generally slender, pale, containing internal parietal gemmules, invisible in the iodine solution (simple or acidulated), but distinct enough in gentian violet. For this kind of *stalks* we refer to the preceding memoir. Then is it not strange that these fertile filaments should be so slender, whilst the severed or truncated (*Bacillus buccalis maximus* and *Leptothrix buccalis maxima* of Miller) appear much thicker and with a strong iodine reaction. We attributed this thickness, or woody state, to the retrocession of the germinal matter, somewhat analogous to the action of the saps in pruning-plants. Our parasite, being a vegetable, or rather a tiny plant, and having to be studied as such, according to Dallinger,* it is natural that the form of its stem should resemble that of the cone. The stalk being, in fact, the proper organ of vegetation, destined to support the upper organs, it is natural that the lower part should be stronger and that it should get thinner towards the top, which, finally, must bear the fruits. It is identical with the process of other plants.

However, whilst this is the general rule, we shall see, as we proceed, that certain fertile filaments become rather an exception, thinning themselves similarly on the top; but afterwards, on reaching the base of the future fructification, they thicken in the shape of a cylindrical club, colouring brilliantly with aniline, but pale like the rest in iodine solution. We shall speak later on of the probable meaning of this swelling of the stalk, recalling only

* Dallinger, *The Microscopical Organisms and their Relations to Disease (Journal R.M.S., 1885.)*

on this subject the other apical swellings, already mentioned and delineated in the preceding Memoir (Fig. 9, *b c*).

To this first element (the internal stem) follows in order of formation the second, of peduncles or ingrafting threads, destined to bear the spores, like the *sterigmata* of many fungi. Such peduncles are very pale, invisible with less powerful objectives, but very distinctly observable (under certain conditions) with the 1/25th power just mentioned. They are short, funnel-shaped, with a point on the stalk and the opening towards the spore. In the young ears, being yet deficient or scanty in the secretion of the viscid substance, the peduncles are more visible; whilst in the older ones they remain more or less opaque.

The spores constitute the third element, and are of globular form ; pale in iodine solution, more or less coloured in the aniline. They are smaller in the younger clusters, on which, for this very reason, the peduncles or sterigmata are more discernible; whilst, the spores becoming afterwards larger and covered with a more viscid substance, the peduncles remain hidden. Besides, the spores are not equally thick in all the fructifications ; but where the spores are thinner, as in Fig. 23, *a*, there the details mentioned are more visible. The little or non-visibility of the peduncles may result, not only from the opacity produced by the abundance and density, or from the heavy colouring of the viscid substance, but even, where this is thin and transparent, from the identity of the index of refraction of the two elements.

The fourth element is the viscid substance or *glair*, which we hold to be the last to form. It proceeds from a sort of oozing or secretion of the stalk or of the spores themselves, for, in the younger ears, with yet small spores, it appears thinner and indistinct.

And these are the fructifications which exhibit a more striking resemblance to real clusters, either in the solution of iodine or when they are very slightly affected by gentian violet. On the other hand, in the ears with an internal swelling of the stalk, the size of the spores, as well as the density and colouring of the viscid substance, reach the highest degree, as we shall see later on. That the viscid substance may proceed from the filament is exhibited by the cited examples, in other species, by Billet, as well as in our parasite, by those filaments of *Leptothrix buccalis maxima* and of *Bacillus buccalis maximus*, which, with the new 1/25th objective, appear thoroughly enveloped in a hyaline sheath. That it may proceed from the spores is shown by the example of the incapsulated diplococci. However, between the exudation of the old filaments and that of the ears there is this difference: that the first has greater affinity for the acidulated solution of iodine, in which it is better discerned, and the second for the gentian violet.

Keeping in view, on one side, these various elements, and, on the other, the degree and different nature of the colouring, we may fully explain the varied aspects of the fructifications.

Concerning the gathering and the preparation of the patina dentaria and its treatment with the aniline colours, or with the solution of iodine, there is little to add to what has been said in the previous Memoir. We gave there the precise rules, in order to obtain from the patina dentaria the greatest possible number of fructifications, taking it before a meal or early in the morning. We found afterwards that the fructifications are more abundant on teeth with a thin film of tartar, particularly in the superior eye-teeth. About the disintegration and colouring of the clods we refer to what has been said. For the acidulated solution of iodine, we placed on the slide, first, a small drop of lactic acid, disintegrating in it afterwards the patina, and lastly adding to it one or two drops of solution of iodine. It generally takes a quarter of an hour to get a proper colouring.

In the preceding Memoir we suggested that the superfluous liquid should be allowed to trickle down from the sides, after applying the cover-glass; but afterwards we discovered that it was better to let most of the liquid evaporate before applying the cover-glass, so as to avoid the wave of liquid caused by the pressure, which might remove many bacteria and bacilli and several isolated filaments and clusters set free from the tiny islands that often supply the most instructive specimens; blotting paper would also take up many of the specimens. To eliminate the air-bubbles keep the cover-glass on edge (straight up) with the two first fingers of the left hand, so as to form an acute angle with the slide, at two centimetres from the preparation; then with a straight needle, held in the right hand to support the glass, and with a bent needle pushing it on the specimen, lower it down gently on to the edge of the preparation, when the air-bubbles will be set free.

If we wish to institute a comparison between the two stains adopted in this work, we may say that gentian violet colours briskly the little spores, but at the same time attacks and obscures the viscid substance, so that,wherever it fully invests, the peduncles or sterigmata are either not seen at all, or may hardly be distinguished. This happens even by applying very little tint. Nevertheless isolated fructifications slightly affected by the tint may sometimes be found in the preparation, and it is just upon one of these occasions that we first detected the peduncles in question. For this reason, the whole image, either of the single fructifications, or of their branching filaments, is better seen in the anilines (see in the previous Memoir the Figures 10, 12, 16); the solution of iodine would hardly give the same result. This solution has little or no action upon viscid matter; therefore, the fructifications assume with it a granular aspect, and if the spores are not too thick it shows the peduncles better; also because the solution of iodine acidulated with lactic acid attacks relatively better the peduncles themselves, whilst the gentian violet invades them less than other elements. Consequently it happens that the solution of iodine better exhibits the complex aspect of the clusters or the truncated sterigmata. (See Fig. 23, *d.)* It has, however, the disadvantage of not satisfactorily allowing the use of powerful eye-pieces, thus limiting the enlargements; whilst in the fructifications stained with gentian violet, the details of structure may be (under favourable conditions) detected even with a No. 6 eye-piece, as is shown in Fig. 24, magnified to 3,100 diameters.

Now from the concourse of these various circumstances, either relative to the age of the single fructifications, and to the more or less thickening of peduncles and spores, or to the quality and degree of the colouring, we are able to obtain images conspicuous in the whole, but with peduncles only partially or not at all visible, or sometimes less conspicuous in the whole, but with quite distinct peduncles. The necessary conditions to the clear vision of the peduncles in question may be summed up in the following series:—*a*, proper optical instruments; *b*, clusters still young; *c*, rather thin spores; *d*, a weak gentian violet; or *e*, solution of iodine. We have already said that the best images are obtained from isolated fructifications fallen from clods in a clear field, and it is our intention in this work to consider the two colourings above mentioned, apart from the use of other tints.

The fructifications in question can be observed by axial illumination or by oblique light. The best images of the clustered forms and of the single sterigmata are obtained by axial illumination by properly adjusting the correction collar, and by centering the iris diaphragm and the Abbé condenser along the optical axis. On the other hand, the general relief of the ears and the position of the spores in six longitudinal series, are better detected by oblique light, by pushing aside the diaphragm and letting it afterwards go round the optical axis, or substituting for it the dark diaphragm, pushed on one side, and making the stage rotate with the upper part of the instrument round the same axis. In the latter case, a better effect is obtained when the ear is horizontally disposed and is *struck* from behind by the pencil of light, almost parallel with the stalk, beginning from the root, so that the luminous rays run along its axis. Then, focussing, the general effect of the six series of spores becomes striking, the whole ear takes a beautiful mulberry appearance, of which it is impossible to give a satisfactory representation.

The figures which we have drawn are, however, sufficient to give a proper idea of the peduncles in question. In Fig. 23, *a*, we have represented a short and young ear, as seen stained with the acidulated solution of iodine, magnified to 1,700 diameters ; in it the spores are thin, and its clustered form is most striking, as we can even perceive posterior rows of little spores. In *c* (same figure) is seen an older ear, thicker and longer (same staining, and magnified 1,170 diameters) in which, however, the peduncles are sufficiently distinct. In *d*, then (same staining and magnification), is drawn a short fragment of a fertile stalk, found by chance in one of the numerous preparations ; only two spores are seen at its base, and higher up the peduncles still thick, but without spores ; the rest of the ear is wanting.* In the above Fig. 24 (weakly stained with gentian violet, magnified to 3,100 diameters)

* The attenuated appearance of this stalk probably depended upon a flowing-out of its sap or germinal matter owing to rupture.

the spores are intact, their funnel-shaped peduncles are visible through the viscid substance, moderately stained. The stalk exhibits several gemmules of reserve.

The facts hitherto given (in support of the arguments expounded or simply suggested in the preceding Memoir), which everybody can verify for himself, are, in our opinion, sufficiently conclusive to warrant us in affirming the existence of a really external sporification or fructification of the normal parasite of the mouth upon six longitudinal series of peduncles and spores. But for a more evident proof, take the specimen drawn in Fig. 25. This specimen, obtained by pure chance, amongst the numberless preparations examined, represents a stalk which, emerging from the *materia alba* or heap, was extending horizontally, and changed abruptly for an upward direction, with the upper part bearing the cluster. It is seen much aslant, but fortunately intact (stained with acidulated solution of iodine, and magnified to 1,700 diameters); it has a round end, with a fine rosette of six rays, formed by six terminal sterigmata, and having the six last spores, probably yet unripe, to their tops.

### FRUCTIFICATIONS BY TEMPORARY SPORES (*Sporids*?) AND BY PERSISTENT SPORES (*Teleutospores*?).

The mycetologists and the algologists call temporary spores, agamic spores, sporids, and conids the spores which are formed without previous fecundation, and are intended by the multiplication of the species in more favourable and immediate conditions (namely, the conids of the *Peronospora*, destined to diffuse the species during a part of the year); whilst the persistent winterly spores, oöspores, or teleutospores, are produced by the act of conjugation, and help to preserve the species from external injuries, and to strengthen their future shoots (like the hybernating spores of the *Peronospora* in the thickness of the hospitable parenchyma, deputed to reproduce the species in the following year). Of the two processes of sporification, one (says De Bary) seems intent upon preserving *intensively* the species through conjugation, the other only to increase its *extension*.*

* De Bary, *Du Développement des champignons parasitaires* (*Ann. des Scien. Nat. Sér. Bot.*, t. XX.).

Of the other processes of propagation through gemmules, sprouts, etc., we have already treated in the previous Memoir, and shall refer to the subject again later on.

All the fructifications of our parasite, hitherto described and drawn, probably belong to the temporary series, with the exception of the specimen given in Fig. 13 of the preceding Memoir, which might be included in the *persistent* series; but from the last researches and other isolated cases, of which we will speak presently, it appears that the same parasite presents also a comparatively scanty number of fructifications of another kind, which, although similar in shape or type, assume special characteristics, so that we are rather inclined to refer them to the persistent series.

We have already mentioned the club-shaped stems, two of which, stained with gentian violet, are seen drawn in Fig. 26, magnified to 1700 diameters. The club is generally long enough, as in *a*; but sometimes we meet short ones, as in *b*, which might be called an incipient phase. In the first named specimen, the club, although complete, is still quite bare, and, to all appearances, represents a hardly-formed expansion, before the sterigmata and the spores have germinated. In fact, in the Fig. 27 (same staining and magnification) may be noticed an ear, on the whole larger and with spores proportionally more conspicuous, exhibiting an internal stem, club-shaped, and brilliantly coloured, quite dissimilar from the pale and slender stems of the first series, but analogous to the bare stem of Fig. 26.

To our knowledge, these ears never reach the length of some others having slender stems (see Figs. 12 and 16), which may depend upon the comparatively limited length of the club-shaped expansions. The spores of such ears are, besides, more conspicuous and pressed together, so as to form on both sides a sort of zone or violet aureola, at a little distance from the stem; and, between this and the periphery, runs a clearer intermediate zone, where the viscid substance is less coloured, but yet capable of disguising the sterigmata. The light proceeding from the condenser must, in fact, cross first the deep violet zone, which is next the slide, then the intermediate clearer substance (the index of refraction of which is identical perhaps with that of the peduncles), and finally the opaque violet zone, which overlooks the cover-glass. At any rate, the result of this optical combination is to hide the peduncles. When in the solution of iodine we come across such ears, the zones become mixed up, and we perceive, on the whole, a triplex series of coloured granules, as is shown in Fig. 13 of the previous Memoir, incompletely represented, which would lead to the supposition that in these ears the secretion of glair is more abundant and thick.*

It appears, besides, that such ears are even more compact and resisting to the mechanical agents of disintegration; also, their fragments always exhibit a cohesion of the single particles, and their brilliant colouring becomes more conspicuous with aniline. We remember having often found similar fragments in sputa; but, not then knowing their nature, we overlooked them. We also remember that some sound ears, or fragments of the same, were found mixed with many minute ears in fructifications upon small flakes of urethral mucus, as we mentioned in the previous Memoir. We may, however, state that, in the preparations of the dental patina, ears of this sort are scanty in comparison with those of the preceding form.

In considering now those more robust and conspicuous forms of fructification, the mind tries, through analogy, to connect them with the process of fecundation, and finds, although indirectly, its existence is confirmed. In the preceding Memoir, we have described some pseudo-inflorescences *in tufts*, having points varying in shape and size, which we held to be future spindle-like bacilli (*Bacillus tremulus* of Rappin) and future comma, or serpentine bacilli, destined, after being dissevered from the stem and becoming free, to perform the functions of spermatia or antherozoids. We gave the reason for such hypothesis, as we also pointed out the likeness of those elements (supposed male organs), with the spermatia of certain well-known fungi, like *Sphærella sentina*, *Fumago salicina*, *Apiosporium citri*, etc.

The *antherozoids* or *spermatozoids* in sea weeds, and the *spermatia* in fungi were considered as elements of fertilisation. The first (*migratory filaments*, *spiral filaments*, *seminal corpuscles*), now

* After presenting this Memoir, we have made further researches (especially on the presence of such ears in the sputum of pneumonia, and on the manner of detecting the peduncles), which we will soon make known.

cylindrical, now ribbon-shaped, furnished with cilia and endowed with spiral movement in various directions, are originally contained in a cellule or male organ (*antheridium*). The spermatia corpuscles, oval or in rods, straight or curved, also very motile, like the analogous forms of the mouth, were, nevertheless, held to have no cilia. They are originally sometimes contained in an appropriate cellule or male organ (*spermogonium*), sometimes they grow freely on the apex of the filaments, and get dissevered simply through disjunction. In our parasite we thought, at first, that the fertilising elements belonged to this last type, and were formed *in a free state* on the stems; but we shall see, by and by, that perhaps even they originate within apposite sheaths, and therefore may be referred to the first type. In general, the spermatia, unable to multiply through fission, have been seen, at times, to germinate on their own account ; one common example of this kind is exhibited in the ergot.

Now, we repeat, the existence of fructifications more conspicuous and distinct from the others (through their large club-like stem and their two zones of colouring, etc.), in the normal parasite of the mouth, would be quite explained, admitting them to possess fertilising elements constituted by spindle-like, comma, and serpentine bacilli, already described by us, and holding the other spores as agamous and temporary. Perhaps the persistent spores in this parasite are destined to go through the intestinal tube uninjured, withstanding the dissolving action of the gastric juices, and emerge into the external world, maintaining in the fæces their vitality for the future diffusion of the species.

As regards the function of conjugation, it may be performed on the already formed filaments, as we see in many other cryptogams, where sometimes the act takes place between two contiguous filaments, the male organ of the one penetrating the female organ of the other ; but nothing prevents us from believing that a fertilisation of another kind may have taken place between the male element (spindle-like, comma, or serpentine bacillus) and the mother spore, before the germination of the fertile filament.

Against these views of ours, a quite opposite hypothesis might be produced, namely, the hypothesis of a commensalism or symbiosis. In such hypothesis the small sporules, and especially the productions by *points*, would not be proper phases of *Leptothrix*, but *parasites* of the *parasite*, or new micro-organisms of another species, come to implant themselves and thrive at the expense of the original parasite in the same way as *Leptothrix parasitica*, Kützing, which with its slender filaments lodges itself on the larger filaments of *Zygnema* and *Cladothrix dichotoma*, as we see in Fig. 21 (stained with vesuvine and methyl violet, then with solution of iodine, magnified to 600 diameters). But, in this way, one might object, for argument's sake, that grapes are so many parasites of the vine on which they fructify. In fact, consulting the figure in question, anyone can see that the secondary parasitical shoots, *c*, *c*, are less thick than our points, which engraft themselves round the stem, like the hairs of a bottle brush. They are not, besides, methodically arranged, but stretch out very much, like stems destined to vegetate on their own account, rather than to complete the organism bearing them. The filaments of *Leptothrix parasitica* implant themselves also upon the stem of *Zygnema*, *a*, *a*, or of *Cladothrix*, *b*, *b*, which feeds them, by means of bulbs, or spores originated in them, *s*, *s*, as the Fig. 22 shows still better (same staining, magnified to 1,600 diameters): spores which are not seen at all at the insertion of our points. And still less the filaments of *Leptothrix parasitica* are seen to drop at last from the central stem, and swim in the medium with the same briskness of our spindle-like, comma, or serpentine bacilli.

### Various Aspects and Forms.

In the preceding Memoir we have spoken of various forms and appurtenances of the parasite in question; we specially point out the bifurcations and trifurcations towards the seat of certain filaments, with tiny radical swellings, like haustoria; and then the more pronounced ones, some at the knots, some at the apex: the latter like small heads. We have already mentioned the other apical swellings (fertile filaments), club-shaped.

We now go on to describe a third form of apical expansions, very scarce, which, provisionally, we shall denominate *sheath expansion*.

Such an expansion, represented in Fig. 28 (stained with gentian violet, magnified to 3,100 diameters), is very pale, has streaks in its contour, not detected with inferior objectives. Its paleness cannot be attributed to insufficient colouring, because the examined form rose on the top of a filament (likewise pale) in the midst of a thick and very pretty tuft of ears brilliantly stained (of the kind shown at Fig. 12), and nearly surpassed them with its point. The external contour is very slender from the base of the expansion up to its point, and between the internal stem and the exterior contour are seen numerous slender, tiny, transverse threads, which are attached to the external sheath by means of more prominent small dots.

At first we could not understand the probable meaning of this structure. That it was not to be taken for the club-like expansions of Fig. 26 was evident from its paleness, being the antipodes of the bright colouring of these, as well as the presence of the internal fine beams. On the contrary, after deeper reflection, we thought we might refer it to other forms already described in the previous Memoirs. One of these forms would be that of the pseudo inflorescences *in tufts* (preceding Memoir, Fig. 14). Comparing the two figures, it will not appear unlikely that the slender threads of Fig. 28 in the present Plate, distended like fine beams between the internal stem and the sheath, growing more and more in a transverse direction, may end by breaking the external envelope and become free *points*, perfect fertilising elements (spindle-like, comma, or serpentine bacilli), at first only free from the surrounding sheath, in order to constitute the tufts of the preceding Plate, and at last becoming disjoined from the central stem, so that they may fulfil their function through that stirring motion, mentioned in the previous work.

The other form, possibly analogous, would be that delineated in the first Memoir (Fig. 2, *n*, lower down), similar to a large bacillus, singularly veined throughout, and for a certain tract having traces of lineal bacilli. Even this is a rather rare form, and we have never found it on the top of any filament, but quite by itself. We have already hinted in the previous Memoir that that veined bacillus might be a sort of receptacle for the future fertilising elements, as an antherid or a spermogone.

Now, supposing that interpretation true, the transmutation from one to the other of the three forms would become clear enough. We should have, in the first form (or *sheath expansion*) of Fig. 28 (present Plate), an antherid or spermogone, hardly shown, and in the second form (the tufts of Fig. 14, previous Memoir) a male organ in full development. The third form (the veined bacillus of Fig. 2, *n*, below, first Memoir) would be an intermediate form to the other two (an arrested form), or an antherid or spermogone, prematurely fallen from its stem, having been unable to attain to the adult form of tuft; and then strayed from its destination and remained, as it were, unripe, or even returned to a neutral condition, which is, we think, common to the severed or truncated filaments, as, having been unable to attain the fructification, they limit themselves to a reproductive function of a lower degree (*fissiparous* multiplication) through the increase of the granules or the lineal minute elements, contained in the interior of their sheaths. If this were confirmed by farther researches, it would lead us to rectify the first supposition about the formation of the *fertilising elements*. They would not form themselves freely on the top of the respective filaments, but within a receptacle or male organ properly so called (sheath expansion).

These are only simple conjectures, aiming at connecting the various forms hitherto described, reconducting the appurtenances of our parasite to the general laws of the cryptogamic flora, and far from pretending to give herewith a full and exact explanation of them. We shall be quite satisfied if the features of the facts we have endeavoured to describe can be proved by further researches.

But, even upon a simple descriptive ground, we should perhaps overstep the limits of a simple preliminary study if we were to dilate longer in the investigation of other particulars, before seeing confirmed and set up the points already demonstrated (such being the most important) by competent authorities. Neither is it our business to solve the question whether the discussed parasite is a fungus or an *alga*. We shall only say that it appears to us to partake of the characters of both families, to thrive as an alga, but to fructify similarly to certain fungi. We shall, therefore, limit our remarks about some apparent irregularities, in the aspect of the described ears, which, through inattention, might pass for true irregularities or anomalies of structure.

In the first place, we refer to some gibbosities or irregular projections, which are met sometimes by the side or upon some ears, which might lead to the belief that the ears themselves are perhaps constituted without any order, or that the series or longitudinal rows of the sporules are not always six in number, but at times more.

Now, one of the more frequent causes of such irregular appearance is very simple. The breaking up of a contiguous ear, and the adherence of that extraneous fragment to the ear that we examine; having frequently verified this occurrence, we considered it superfluous to draw a similar specimen on our plate. The other case, less frequent, is drawn in Fig. 29 (saturated with acidulated solution of iodine, magnified to 1,700 diameters). Here it is not the superposition of an extraneous fragment, but the folding up of the ear itself. In the figure referred to, the third superior and the point *b* of the ear are turned up and pressed back upon the middle third, but in a direction somewhat oblique to its axis, so that, at first sight, or with inferior objectives, they simulate a gibbosity. But focussing with the fine adjustment, it is easily perceived that there is, in reality, a folding due to a mechanical cause, and accidentally rendered even more pronounced by the pressure of the cover-glass.

We do not speak of a third apparent irregularity, which might deceive us only when using inadequate optical means—we mean the accidental apposition of extraneous cocci, of bacteria and comma bacilli around some ears, as shown in Fig. 23, *b*, *b*. Under lower power objectives, such cumuli or groups, especially if more conspicuous, may, in fact, simulate real protuberances.

There is, however, a special apposition of cocci and bacteria, sometimes visible on certain ears; perhaps, those remained longer with their points in contact with the labial mucous, and there became incrusted with the above bacterial elements, through a cement of viscid mucus. Such ears, in fact, are never augmented on the opposite side, but only on the top, like a very oblong pear; and the increase, gradually narrowing itself, seldom goes beyond the half of their length (Fig. 30, stained with gentian violet, magnified to 1,170 diameters).

When they reach the first colourising stage with the gentian violet, we can easily perceive there is in reality a sort of cap (at first, more pale and granular) constituted by cocci and bacteria, only slightly coloured, in various layers, towards the point *a*, and sloping towards the half, *a'*, *a'*; whilst the ear, *b*, *b*, with its sporules, is seen brightly coloured in the interior. In the second stage, even these adventitious cocci and bacteria become coloured, and the cap is no longer distinguished from the internal ear.

## CONCLUSION.

We have seen how Billet describes the evolutionary cycle of the Bacteriaceæ (which would constitute for our parasite only the inferior cycle), and that his remarks mostly agree with those we have made on the same parasite. His interpretations of the various phases of that cycle do not differ from ours, excepting in what affects the production of bacteria included in filaments, which Billet considers are real endogenous spores; and we cannot positively deny that such is the case in the four species studied by him; whilst in our parasite they ought, in our opinion, to be held as simple gemmules of reserve.

But from the exposition of the facts in our previous Memoir, and confirmed in the present one, it clearly results that the evolutionary cycle, so nicely delineated by Billet, cannot include all the morphological phases of our *Leptothrix racemosa*, but only some of them. In this parasite, besides that first cycle which we call *inferior*, there is another—the *superior*, which comprises the organs of genuine reproduction and fructification. Finally, together with these two *normal* evolutionary cycles, there is another one—*accidental*, called *virulent*, in which (according to laboratory experiments) it seems that certain elements, derived from the parasite itself, may, as Pommay says, develop themselves in the sense of virulence.

The first two cycles would, therefore, constitute the *morphological series*, and the third, or virulent, cycle would, in modern language, constitute the *biological series* of our parasite.

MORPHOLOGICAL SERIES.—*Inferior Cycle.*—The inferior cycle embraces the following phases :—

I.—*Phase of Vegetation.*—The characters of this phase are those assigned by Billet to the filamentous state, only that he considers the intertwined state (*état enchevêtré*) as a distinct and later phase; whilst we believe that it, in our parasite, accompanies the filamentous state, in the same manner as the mycelium or creeping vegetation in fungi. In other words, the intertwined state is even posterior to the isolated filaments which lead a wandering life in the liquid substrata, being unable to attain a more vigorous and stable one; but it does not constitute a distinct phase, as it is quite natural that, when the passage to the superior phases is precluded (*aërial* vegetation) for want of a fit soil, the filaments intertwine and drop to the bottom, without being able to spread like the mycelium of fungi. However, under favourable conditions, a kind of mycelium (a more complete phase of the entangled state) may be formed, giving birth to an aërial vegetation, as may be specially observed in the patina of the tongue and in the deep strata of the patina dentaria.

II.—*Phase of internal gemmulation or budding and dissemination.* This partly corresponds to the dissociated state of Billet, through the disarticulation of the knots of the single filaments, or through setting free the included bacteria; and it is clear that the unstable condition of the nutrient medium continuing, and consequently the passage to the superior phases being prevented, no other way of perpetuating the species is left to these micro-organisms than the inferior reproduction or simple multiplication by shoots and gemmules. Looked at in this way, this phase would even comprise that held by Billet as endogenous sporulation, but which are, at least in our parasite, gemmules of reserve, properly destined to the multiplication of the species, in a neuter state, through simple fission of the elements, when the genuine reproduction, by means of seminules or spores, is not possible, or when the fertile filaments have been dissevered or cut by mechanical injuries.

III.—*Protective phase.* This phase fully corresponds to the zoöglœic state and to certain conditions of the dissociated state of Billet, as we have pointed out in the first paragraph. The presence of such forms has been undoubtedly detected in the mouth, and even within the relative epithelia. They would appear to be a kind of reserved fund, preserved in case of any alteration of the future conditions of pabulum and surroundings, as the author properly says. We shall speak by-and-by of the relationship of this phase with the diplococcus of pneumonia.

Here Billet would end the evolutionary cycle; for us, on the contrary, these first phases would only constitute a cycle, at times preliminary, at times succedaneous to the second or superior cycle. The varied elements of the first cycle, being taken separately and held as special beings or complete living individuals (filaments, bacilli, bacteria, and cocci), are in reality only particles, trunks, organs, series of cellules, or cellules endowed mostly with fissiparous multiplication, but destined to constitute a more complex organism.

SUPERIOR CYCLE.—This comprises the phases of the properly called life of reproduction, and these phases are three.

IV.—*Agamous Fructification.*—Referring to the general laws of multiplication of the phanerogams by means of bulbs, tubers, shoots, or buds, and of their genuine reproduction by means of seeds, it should be borne in mind that with transplantation are transmitted the accidental modifications brought about by domestication, grafting, etc., which, in the long run, may end in the degeneration of the plant; whilst with seeds, on the contrary, the species reverts to the natural vigour of the wild state.

Now, we incline to believe that something similar may happen even with cryptogams. Probably in those which have, besides a fissiparous multiplication, a true reproduction through spores, the sporulation will mean that the species resumes its native vigour, in spite of the attacking or enfeebling causes which, as in our parasite, may impair its vigour.

The seminule or spore may germinate without previous fertilisation, and (as we have already said) we believe this may happen more frequently in our parasite. In the species studied by Billet, this external sporification or fructification was wanting, and it is remarkable that the cocci, which are found abundantly in the buccal contents, were likewise wanting. It remains to be seen whether this want of fructification is really the rule, or simply a consequence of the nature of its pabulum and the material upon which Billet based his researches. Under other conditions, upon media not only stable, but favourable to the production of a mycelium-like growth, a true and proper fructification might, even in the above-named species, take place.

In our parasite, the conditions indispensable for the fructification are :—*a*, The solidity and nature of the soil; *b*, the protection against attrition ; and *c*, moisture of the saliva.

V.—*Organs and Fertilising Elements.*—By the side of the agamous sporulation we have, in many cryptogams, that of conjugation. This admits of male organs and fertilising elements or spermatic threads, of which we have already spoken. In our parasite, likewise, we have male organs and fertilising elements. The male organs would first show themselves in the state of young spermogones or antheridia (or of spermogones or aborted antheridia, not developed or reverted to the neuter state), and afterwards in the state of tufts of ripe pseudo-inflorescences, proceeding from the first type. On the other hand, the fertilising elements would be constituted by the spindle-like, comma, or serpentine bacilli, already formed in the described organs, and finally set free, through the disarticulation of the tufts already mentioned.

VI.—*Conjugated Fructification*—*i.e.*, that with clavated stem, a dual zone of colourisation, clusters, or more conspicuous ears, more bulky and compact spores, destined, perhaps, to cross uninjured the alimentary canal, and to remain alive in the fæces, withstanding the dissolving action of the gastro-intestinal juices.

Nature, as we see, has been prodigal to this parasite, by its various manners of multiplication, adapting each of them to this or that condition of the nutrient substratum, in order to preserve and multiply its species, in the midst of numberless and very varied difficulties. As soon as a higher phase is precluded, or a nobler element is thrown back, by external injuries, to an inferior degree, it does not stop from disseminating everywhere particles apt to germinate and spread *extensively* the species when it cannot do it intensively. We are reminded that Nature has even wished to endow this tiny plant with such a tenacious life, that its elements on the human teeth cannot be destroyed for centuries, as exhibited by the tartar on the teeth of Egyptian mummies (*vide* the preceding Memoir).

If here ends, at least provisionally, the *Morphological Series* of our parasite, it only remains to us to say two words on the *Biological Series* in the modern sense, or, rather, on the pathological phenomena, assigned to some of its forms, or to specific bacteria similar to the latter.

Biological Series.—Under this title we comprise but three forms: the *Pneumococcus*, the *Bacillus* of Koch, and the *Gonococcus* of Neisser, already demonstrated, according to our views, in the two previous Memoirs.

I.—*Pneumococcus.* Few now hold that the pneumococcus is a specific bacterium, arising externally. It is generally considered to be either an habitual germ of the mouth (*Micrococcus of salivary septicæmia*), or, in common with Pommay, a saprophytic bacterium evolving itself in the sense of virulence. Perhaps it will in time be known as a simple dissemination of the zoöglœic phase of our parasite, following on the formation of the pulmonitic exudation, as its greater abundance in the last stages of pneumonia, its presence in traumatic pneumonia, and other evidences will prove it to be so. But even admitting that the evolution in the sense of virulence may take place in the mouth and not in the respiratory organs affected (as regards virulence, which may be inoculated); admitting that it has preceded and not followed pneumonia, we cannot necessarily infer that the disease proceeded from this coccus. But even the colonies of these diplococci, when repeatedly transplanted in other culture media, or even the media themselves, may become contaminating through inoculation, this circumstance may also occur with other salivary bacteria (for example, *Bacillus crassus sputigenus*), and might be interesting in experimental pathology; but, botanically speaking, it does not implicitly imply a separate specific entity. We repeat that we do not impugn, but rather try to conciliate the results of the inoculations with those of the morphological research.

II.—*Bacillus of Koch.* Our remarks concerning this bacillus are very similar, and we hope at a future time to devote a special Memoir to it. The reasons which induced us to believe the bacillus in rosaries to be a dissemination of the small spores of *Leptothrix* spread over the tubercular spots, and the rod-shaped bacilli as proceeding from other elements of *Leptothrix*, have been already given in the preceding Memoirs. Their greater dissemination in phthisis than under other conditions may depend upon different causes, and partly, perhaps, from the following simple reason :—Breathing through the nose, as we generally do, only a few germs of a purely buccal origin are inhaled ; but the position becomes altered when we breathe through the mouth as well, as happens in phthisis, because of panting or burning heat :—

> " . . open'd wide his lips,
> Gasping as in the hectic man for drought,
> One towards the chin, the other upward curl'd."
>
> —Dante, *Inferno*, XXX., Cary's Transl.

Now, the small sporules of *Leptothrix* exclusively originate from the patina dentaria, and without that particular form of breathing (*i.e.*, by the mouth) they cannot gather in any great number into the air-passages. Many also, in sleeping, breathe likewise with their mouth ; but in the normal conditions, however, the ciliary action continuing unhurt through the air-passages, the inhaled leptothrical elements cannot reach the pulmonary tissue, but are instead thrown out and expectorated with the sputa, as stated in the other Memoir.

III.—*Gonococcus of Neisser.* Probably, says Pommay, a normal bacterium of the urethra is the progenitor of the gonococcus of Neisser ; it is useless to repeat here that relationship. Let it suffice to record the fact that we have detected in the urine numberless gonococci, in a case of carcinoma of the bladder, independently from any gonorrheal contagium whatever.* According to the morphological characteristics, the gonococci in question do not differ from the ordinary arched diplococci, and therefore can be held, till proved otherwise, to be derivations from the state of sarcina (one form of the zoöglœic state). As regards the manner by which the leptothrical elements penetrate from the external genitals into the urinary passages, as well as the presence of analo-

**Atti della R. Accademia Medico-Chirurgica di Napoli*, tomo XLIII., 1890.

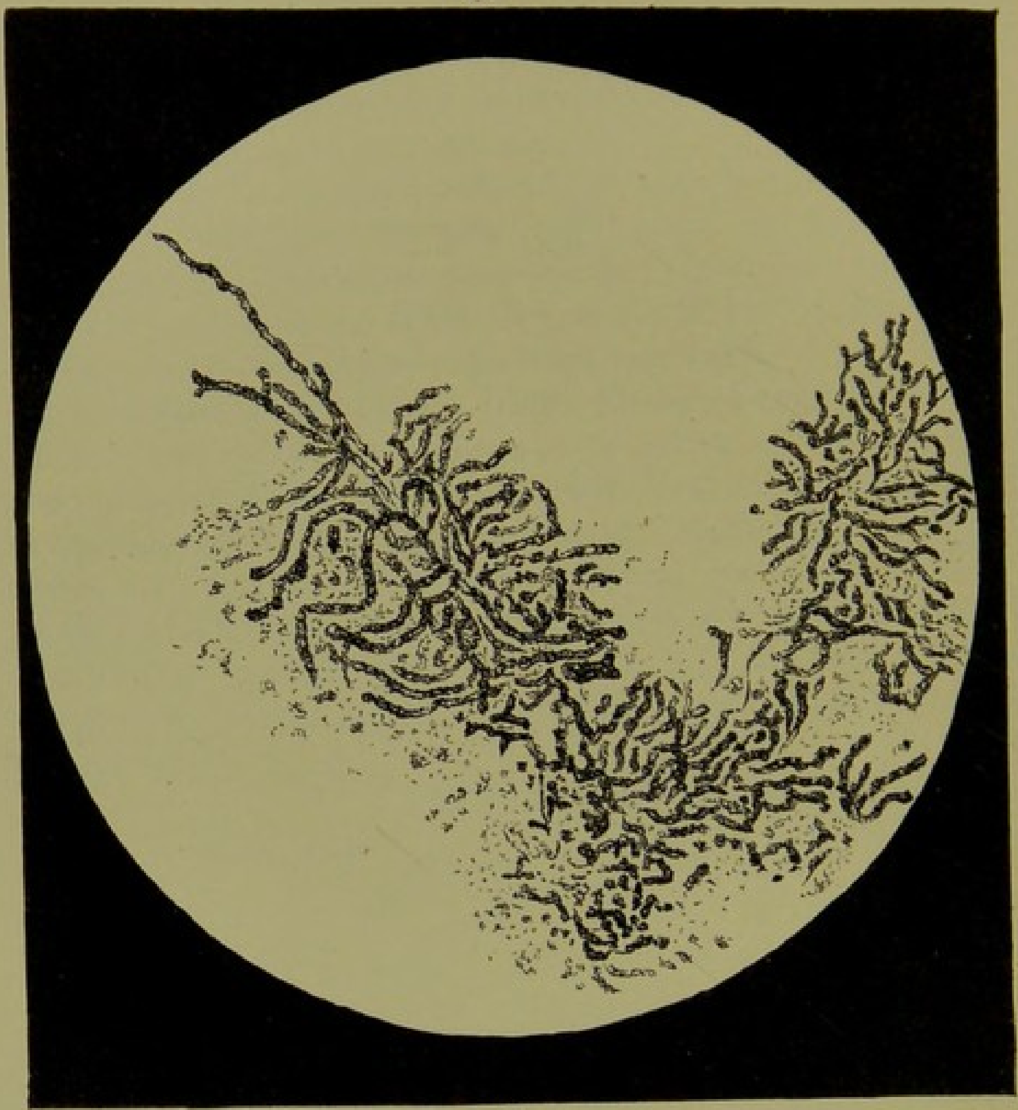
gous forms (curved diplococci) in the contents of the mouth, in sputa or the middle ear; and also as regards the discovery in the urine or sperm of capsulated forms (forms analogous to pneumococci), we refer to the previous Memoirs.

From the morphological sketch we have just given, nothing perfectly absolute and incontroverted is to be inferred, either for or against any previously acknowledged systematic view; but perhaps few people will doubt that, in the field of modern bacteriology, much still remains to be done, and a great deal of discussion, combined with careful study, will be required before any definite and satisfactory results will be arrived at concerning the pathogenesis of infectious diseases (we are, of course, now alluding to those cases which are strictly clinical). Many will, we are sure, agree with us that, with regard to bacteria, our knowledge is so limited that no method of investigation will appear superfluous in order to study aright their genesis and properties in order thoroughly to understand and appreciate their function in our economy.

F. Vicentini, Corresponding Member.

*Chieti; May, 1892.*

Fig. 31.



x 400.

Fig. 32.

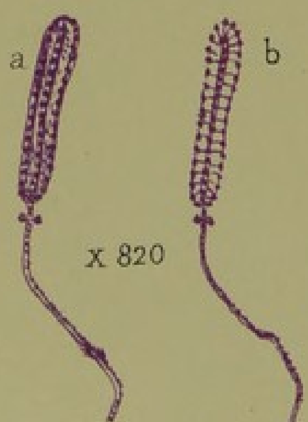
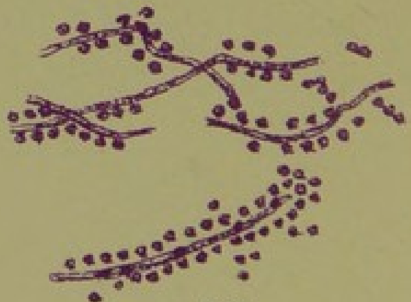



Fig. 33.



x 820

# Bacteria of the Sputa and Cryptogamic Flora of the Mouth.

*APPENDIX TO THE ENGLISH EDITION.*

TRANSLATED BY E. S.

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SUMMARY.

Reasons for writing this Appendix. Doubts about the Bacterium hypothesis (the views of *Jaccoud*, *Middendorp*, *Pettenkofer*, and others). Hypothesis of the author on the action of Bacteria upon the human and animal economy (in the *normal state*, in *disease*, in *laboratory experiments*). Some further remarks on the morphology of *Leptothrix*.

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## Reasons for Writing this Appendix.

IN making this translation, I was, at first, tempted to recast the preceding Memoirs into a single work, shorter and more synthetical; but I have chosen to re-introduce them in their original form and arrangement, wishing to keep unchanged the itinerary, so to speak, in stages, which I have followed up without a preconceived plan, but simply guided by my own clinical investigations. In the present Appendix I shall endeavour to answer, as briefly as possible, a few objections, and give some more particulars on the morphology of *Leptothrix*.

The control exercised by other investigators upon the morphological phases described by me will be confined to three distinct objects :—

1.—If my observations are exact, *Leptothrix buccalis* possesses real organs of fructification, and probably also organs of fecundation, similarly to a fungus or an alga of a more complicated structure. Consequently, the first point of control will be to ascertain the existence of such organs. From the preface, which Prof. Miller has kindly prepared for this edition, it is apparent that he has had no great difficulty in finding the "grape bunches" appearances described by me. Of such forms he drew the photomicrograph, reproduced in Fig. 31 (magnified to 400 diameters).

Likewise, they have been detected by Mr. J. H. Mummery. In his paper before the Odontological Society he says :—" I certainly detected appearances very similar to those organs which Dr. Vicentini compares to grape bunches, and made drawings of them at the time. The regular lines of rounded bodies, which the author considers to be spores, were plainly seen, as well as the central stem ; but I was unable to see the minute peduncles, by which he describes the spores as attached to the central stem. The club-shaped appearances of these bodies were also very evident ; but so far I have been unable to see anything that I can identify as corresponding to two forms of the male organs, although I do not consider I have done justice to the author in my search for these, and shall renew the attempt. The forms which I saw certainly seem to be too regular in shape to be explained as an accidental accumulation of granules or micrococci around an isolated filament (an explanation which has been given by some observers)." *

Of the existence of forms by *ears* or *grape bunches* there appears, then, to be no doubt ; it remains only to be seen if, as I detected with a 1/25th inch immersion lens in such ears, real peduncles or *sterigmata* exist, and whether the other forms, considered by me as male organs or fecundating elements (not to speak of less important forms) are as I described them.

2.—Let this first question, relating to facts, be once solved, and there naturally arises that of their interpretation. Are the above-named ears true fructifications and the tufts real male organs ; or is it, in both cases, a question of *commensalism* or *symbiosis*, or some other phenomenon ?

Some correspondents have expressed to me their doubt that the ears in question might only be a particular zoögloeic form growing into the internal dental tubules, already described by Zopf.†

But everybody can see that between the two there is not a single point of analogy in the structure. The figure of Zopf,

* J. H. Mummery, *Transactions of the Odontological Society of Great Britain*, Jan., 1894, p. 77, partly reprinted in the *Lancet* Feb. 24th, 1894, Vol. I., pp. 487—88.

† Zopf, *op. cit.*, Fig. 4, Miller, "Der Einfluss der Mikroorg. a. d. Caries," etc. (*Klebs' Archiv*, XVI., 1882, Fig. 7).

reproduced by Miller in *Klebs' Archives*, represents a simple heap of cocci and bacteria, mechanically modelled within the dental tubules, and not a production germinating upon the *free surface* of the teeth. It has no central stem, sterigmata, or peduncles; its single elements are heteromorphous, having at one end bacilli, in the middle bacteria, and at the other end cocci; whilst the *ears* bear round, uniform spores in six longitudinal rows. Finally, the heaped-up elements in the dental tubules are more bulky and easily discernible in their normal state. On the other hand, the spores in question are very much more minute, and cannot be distinctly detected without being properly stained.

With regard to the process of eventual fecundation, which I have foreseen in the *Leptothrix*, it will not be inopportune to repeat what Dr. Dallinger writes on the possible conjugation of bacteria :—" Nothing like 'conjugation,' or any other form of sexual generation, has yet been witnessed in any bacteria; and until such shall have been discovered, no confidence can be felt that we know the entire *life-history* of any type. As it seems unquestionable that among the higher fungi 'conjugation' often takes place at a very early stage of growth, it seems a not very improbable surmise that the 'granular spheres' observed by Prof. E. Ray Lankester, in his *Bact. Rubescens*, may be a product of conjugation in the micrococcus stage of these organisms." *

3.—Having solved these two questions (namely, of the facts and their interpretation), we may with some reason enter the field of the third question, or of the deductions to be derived from the exhibited facts, concerning the single isolated bacterial forms of the buccal cavity, and see whether such forms really constitute as many independent species, or if many of them are not rather simple disseminations or rudiments of one or more superior species of micro-organisms.

Now, it so happens that in looking at my researches this last part has had the first consideration, particularly in relation to pathogenic bacteria. Prof. Miller, besides, attributes to me as a firm and full conviction what I simply suggested as doubtful and

* Carpenter, *The Microscope and its Revelations*, 7th edit., by Dallinger. (London, 1891, p. 588.)

hypothetical—viz., that a great proportion of the bacteria, which is prolific in the mucous membranes, might proceed from a common stock. But I emphatically state that a definite opinion upon that subject would at the present moment be premature.

Prof. Miller and Mr. Mummery hint at the revolutionary character of my views; but others, long before me, had advanced the problem of an eventual simplification of the bacterial species. The opinions of Hallier and others upon this subject are well known, and Mr. Mummery has opportunely recalled the opinion of Naegeli, as well as a passage in conformity with the same, from the article, "Fungi" of the *Encyclopedia Britannica.**

Finally, we must repeat the hypothesis of Brefeld concerning a possible derivation of bacteria from *fungi of a higher order*, which may have *lost their organs of fructification*, so that their successors could multiply by no other manner than by fission.†

My reason for pointing out the problem of the classification of the species of bacteria was simply to anticipate the objections of specialists against my experiments, and I will now endeavour to explain away some of those objections. On this point Mr. Mummery recalls the fact advanced by Prof. Miller, that "a pure culture of the micrococcus of the sputum septicæmia, one or two days old, will invariably kill a mouse or a rabbit; whereas a pure culture of some other bacterium from the same mouth, under exactly the same conditions, may have no action at all. This (he adds) seems very extraordinary if they are all derived from one single form in the mouth." ‡

We might admit a distinct specific entity for this or another definite form of bacteria decidedly virulent, without admitting the specific entity of all the remaining forms; but in the second Memoir I have already referred to the insufficiency of peculiar vital action as forming a basis for the classification of the bacterial species. The distinct characteristic of the other cryptogamic species (I said) is to be found in the fructification or the completed series of the various

* Mummery, *loc. cit.*, pp. 79, 80.

† Brefeld, *Untersuch. ü. d. Spaltpilze, Bac. Subtilis. (Gesell. Nat. Freunde*, Berlin, 1878, *Schimmelpilze*, Heft. 4).

‡ Mummery, *loc. cit.*, p. 80.

morphological phases of each. No one would pretend to classify an *alga* and a *fungus* from observation based upon experiments of the vital action of their single elements on their hosts. But, departing from the ordinary rule, if we wish to assume such a distinct criterium for the classification of certain species of bacteria, simply because their natural history is at the present to a great extent unknown, it does not follow that the same should be entirely overlooked.

Many parasitic fungi, which in various ways infest their hosts, assume different phases and forms, at times changing upon the one or the other. These divers forms, formerly believed to be as many species, are often simple modifications or particular conditions of a single parasite, as happens for instance in the well-known alternation of generations.

The alternation of generations (as, for example, in the *Uredineæ*, '*ergot*,' etc.) exhibits many examples of this kind. Thus, the *Puccinia graminis*, the cause of the *mildew* in wheat, only represents a first phase (the *teleutospore generation*) of an alternating fungus. The same fungus, passing afterwards from the wheat to the barberry, undergoes a second phase (*Æcidium Berberidis*, or the *æcidiospore generation*). From this a third generation is produced, which returns to infest the wheat, taking up then two distinct forms, one being a reversion to the primitive phase (*Puccinia graminis)*, and the other, altogether different, the *Uredo form*.

Further, the recent history of the *Mycetology* exhibits, at every step, a progressive working of simplification in this sense. One half, perhaps, of the old species of fungi has already disappeared; nor would it be surprising if a similar simplification happened even for the bacteria forms. Are not bacteria even lower in the cryptogamic scale, more slender, and more exposed to the agents of destruction? No wonder, then, that they possess a greater faculty of adaptation and a more extended polymorphism.

In the case of the micrococcus in the *sputum septicæmia*, in spite of the virulence of its cultures, we are inclined to deny its specific entity, and hold it to be a phase (a capsulated zoöglœic form) of the *Leptothrix itself*, considering its close analogy with the proper capsulated forms of other and altogether different species of bacteria—viz., *Cladothrix dichotoma*, *Bacterium Bal-*

*biani*, *Bacterium osteophilum*, and *Leptothrix parasitica* (Kützing), see Figs. 17 and 18.

Prof. Miller has, on this point, dissociated himself from a fact apparently unimportant—viz., the change of state, a change which by itself may, in certain cases, produce bio-physical and bio-chemical effects analogous to those of a real change of species. It is, then, by overlooking the standard of the particular morphology, that laboratory experiments may lead us erroneously to make an unlimited number of species.

Consequently, the greater number, if not the whole, of the isolated bacterial forms in the mouth could be related to *Leptothrix*. If the minute cryptogamic plant which I described exists, where shall we place its various particles, detached through ripeness or removed by injuries (as *spores*, *sporids*, *gemmules*, *chains*, *filaments*, enclosed bacteria, and male elements), if none of the bacterial isolated forms belong to it ?

It is, I think, easier to suppose a common derivation of these isolated forms from the above particles of the same plant (like fruits or leaves fallen from the same tree) than the special and altogether distinct origin of them from without.

With regard to the experiments of culture and inoculation, far from minimising their importance, I intend to show later on that, being considered within their proper limits, although interpreted in a different way, such experiments may entirely coincide with my opinion of a possible simplification of the bacterial species. True, the bacterial science is still too modern to over-reach a whole system of etiology, and generally the systems have never thoroughly succeeded in medicine. Thinking of the limitation of our minds before the mysteries of life, we may entertain the doubt as to whether the intimate reason and essence of diseases in general, and of the infectious ones in particular, might be accessible to our knowledge, as the greater number of bacteriologists pretend. But, however, I do not presume to minimise the importance of such studies. I have already given in previous works the opinions of several authors upon the limits to which the study of bacteria in medicine may be applied. Now, I will add a few more recent extracts, advising the reader to refer to the writings of Prof. De

Giovanni of Padua upon the relations which exist between the laboratory and the clinic.*

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**Doubts respecting the Bacterial Hypothesis.**

*Jaccoud's Views on the Spontaneity in Microbian Diseases.*

Prof. Jaccoud in 1882 concluded one of his lectures in the following terms:—It is its source of origin, which makes the bacterium infectious, and not any efficiency attached to it simply as a bacterium. The infectious properties of bacteria are properties borrowed from the soil upon which they have lived, and which they can keep up from generation to generation, even upon artificial cultures.†

He observed that the traditional empirical etiology of tuberculosis, as well as of other infectious diseases, maintains all its value confronted with the bacterial doctrines; that pneumonia, for instance, and endocarditis are not always due to microbes coming from without, but rather to an *inward infection*, following upon a diminished resistance of the organism to microbes, which it bears itself: indifferent pathogenic microbes, capable of provoking affections of various seat and form.‡

In 1888 he resumed the argument of such auto-infection, attributing it to pre-existing germs in the organism, harmless, but which, owing to external circumstances (of a corporeal or cosmical order) may become pathogenic.§

In 1892 he deplored the tendency that modern bacteriology had of suppressing medical etiology through three erroneous suppositions:—(1) By considering the pathogenic microbes as extraneous to a healthy organism; (2) by considering them as the

* *De Giovanni, sulla tisichezza pulmonare*, Naples, 1882. *Uno sguardo alla batteriologia*, 1886—87. *La linfa di Koch, communicazione R. Istituto veneto*, 1891, pp. 24—28. *Morfologia del corpo umano*, Milan, 1891, pp. 113—119. *Commentari di Clinica Medica*, Vol. I., Padua, 1888, pp. 36—62, Vol. II., Padua, 1893, pp. 75—177.

† Jaccoud, *Cours de Pathologie*, Leçon du 11 Nov., 1882. Paris, 1893, p. 15.

‡ Jaccoud, *Clinique médicale de la Pitié, pour* 1883, 1884, Paris, 1885, p. 23, *et pour* 1885—86, Paris, 1887, pp. 94—98.

§ Jaccoud, Leçon du 5 Mai, 1888 (*Bulletin Médical*, 11 Nov., 1891, p. 1034.

originators of *effects* which are *always identical*; (3) with reference to the supposed immobility of the species of bacteria. The pathogenic microbes, he says, are not always extraneous to a healthy organism; on the contrary, a large number of them normally lodge in it. The same disease, for instance, endocarditis, may be induced by various microbes, and these, likewise, are not unchangeable, but assume various forms. Thus, if certain pathogenic microbes normally exist in the healthy organism, this fact demonstrates that the diseases connected with them arise *in us* and *through us*, else the disease would be a normal condition and the healthy state a myth. The microbe, for Jaccoud, means only the *instrumental cause* of the disease; the *how* and not the *why*.

Nevertheless, micro-biology presents, according to the author, quite a new character; the *transmissibility* to others of a disease originally autogenetic from some common injurious cause—a cold, for instance, although even this fact is, in its turn, subject to the law of a morbid spontaniety.*

Amongst the microbes which in a harmless state thrive in the human organism, Jaccoud places the *Pneumococcus*, *Pneumobacillus*, *Streptococci* and *Staphylococci*, the *Bacterium coli commune*, and numberless bacteria that swarm upon the skin and the mucous membranes. He affirms that there is not a single one of these microbes which may not become pathogenic in the affections of the bowels or membranes, in pyæmia, or in erysipelas, rheumatism, etc. The *Bacterium coli commune*, for instance, and its congeners may enter into the autogenesis of the intestinal affections, causing catarrhal burning, and ulcerous affections, and even typhus or cholera infections, cholera itself being not always characterised by the presence of *Bacillus virgula*.

Then we are in danger of having a plurality of microbes for the same disease, and a plurality of diseases for the same microbe. This is the rock, says Jaccoud, against which the supposed specific character of the microbic diseases is likely to be wrecked.

The characters of a really specific disease (as poisonings

* Jaccoud, *Note à la Traduction de Graves*, first edit., Paris, 1891, p. 142, and *Clinique Médicale de la Pitié*, 1886—87, Paris, 1888, pp. 52—55.

by animal venoms, eruptive fevers, syphilis, etc.) are, according to the author, five:—Exteriority of the cause; unity of cause for each disease; unity of effect for each cause; immutable relation between cause and effect; and constant reproduction under the same species. Now, he concludes, it is manifest such are not at all the characters of the microbic diseases; whilst, on the other hand, there is the fact that the greatest part of the *diseases truly specific* (*eruptive fevers, etc.*) in any microbial character is hitherto wanting.* Jaccoud means to indicate that the diseases truly specific (eruptive fevers, etc.) present no peculiar bacilli as their bacillar characteristic, whilst the contrary should take place, according to theory.

*Middendorp's Remarks on Tubercle.*—From the time that the surprising researches of Prof. Koch upon the bacterial characters of tuberculosis were made known, there have been opponents to his theory. Prof. Spina, who was working with Prof. Stricker, opposed it by observing that if the bacilli of Koch should be the true cause of tuberculosis, they ought to *precede* the formation of tubercular nodules. Let this precedence be disowned, and the principal base of the new theory falls to the ground. Now, he stated that he had never met with bacilli in the young tubercular nodules, and that the bacilli found, by a further observation, in the decayed tubercles and in sputa not only were mixed up with a mass of other bacterial forms, but they did not even possess any truly distinctive characters.†

Dr. Beale had already expressed a doubt respecting the existence of true giant cells. He considers those forms as heaps resulting from the development and subdivision of nuclei (bioplasts) in a *formed material* or stroma, slightly consistent and continuous in its mass; and he adds, besides, that such cellules are often wanting in many specimens, even typical of tubercles.‡

* Jaccoud, *De la Spontaneité dans les maladies microbiennes. Leçon d'ouverture (Bulletin Médical*, 16 Nov., 1892, pp. 1407—10).

† Spina, *Studien über Tuberculose*, Wien, 1883. See also *Wiener Med. Presse*, 1883, No. 19. Concerning the Experiments of the same author upon the colouring of the bacilli in question, and the observations of Coppen Jones and others, see later on.

‡ Beale, *op. cit.*, pp. 330—31.

Charrin pointed out that, from the early discovery of Koch, numberless cases of acute miliary tuberculosis had taken place, in which the characteristic bacilli within the tubercular nodules were wanting; and this want was, from the beginning, attributed to imperfection in the method of investigation; that analogous facts had happened later on to which the same explanation could not apply. He afterwards quoted a case of acute miliary tuberculosis in an adult, in which were found numerous tubercular granulations without caseous degeneration or ulceration, and likewise without the bacilli of Koch; nor could such bacilli be discovered in any way, even in the inoculated guinea pigs.*

Kuskow was also unable to find specific bacilli in the *primitive acute miliary tuberculosis*, when the granulations were isolated within healthy tissues. He discovered them in cases of *secondary acute miliary tuberculosis*, consequent upon a chronic tuberculosis. He affirmed that the acute miliary tuberculosis does not at all proceed from the bacilli of Koch, and that such bacilli only appear there second-hand, proceeding from pre-existing tubercular nodules.†

But the strongest opposition to the doctrine of Prof. Koch, on the ground of pathological anatomy, is actually that of Prof. Middendorp, of Groningen. He has for several years maintained that the bacilli of Koch are but a simple dissemination of buccal microbes, as he had never detected them in the young tubercles, which were not yet in communication with the bronchia. When the lymph of Koch was so highly proclaimed, Middendorp thought fit to divulge his researches; but I shall not refer here to that keen contest. Briefly, Middendorp, from the figures exhibited by Koch, shows that the latter has found bacilli in tubercles only in a retrogressive phase, and already in communication with the air-passages, and not in tubercles which were either partially or fully developed, but still healthy and grey and isolated. The author then mentions that he has never come across any such bacilli, either in young tubercles, in *yellow, caseous* tubercles, or in cavities not communicating with the bronchia. This would prove, according to him,

* Charrin, *Comptes Rendus de la Société de Biologie*, 12 Oct., 1891.

† Kuskow, *St. Petersburger Med. Wochenschr.*, 1891, No. 36.

that the relative bacterial germs, instead of preceding the evolution of the tubercle, are transplanted by second hand, and a second time in the tubercular mass in decay from the nursery, existing in the mouth; and consequently they have nothing to do with the cause of the disease. Middendorp likewise impugned the existence of true *giant cells*, for which he was rebuked, although he was not the first to doubt the true or supposed origin of them.*

Middendorp treated largely upon the experiments of Villemin and Waldenburg, about the inoculation of tuberculosis in animals, adding reports of numerous inoculations, practised by himself upon dogs.

To the censures made to him Middendorp replies a second time, more particularly explaining the points relating to the origin of the bacilli of Koch. "Here is (he says) the quintessence of the problem concerning the doctrine of Koch on the bacillary origin of tuberculosis, on the biological properties of these microbes that he pretends to have discovered, and on the curative method founded by him. I deny the existence of bacilli in sound tubercles of recent formation, *yellow* or *caseous*, as Koch and the bacteriologists of his opinion would make us believe; and likewise I deny their presence in the same cavities, until these enter in communication with the *bronchia*. Then the situation changes; but the bacilli found in such *tubercular cavities, communicating with the bronchia*, or in the sputa, are *common bacilli of buccal mucus*, having, as such, reached the morbid seat, or in a state of germs. In order to place our faith in the theory of Koch, that such bacilli are in etiological relation with tuberculosis, he will have to show them to us, not in the expectorated matter nor in the tubercular mass taken from the walls of a cavity or cavern in communication with the bronchia, but in *tubercles in a state of development*, or in tubercles already fully developed, but still *fresh, grey, raw, and healthy.*†

* Middendorp, *Le Remède de Koch, sa valeur, etc.*, Paris, 1891. *Der Werth des Koch'schen Heilverfahrens gegen Tubercolose*, Emden and Borkum, 1891.

† Middendorp, *Nouvelles études concernant les bacilles tuberc., etc.*, Paris, 1891. *Weitere Mitthei lungen, ü. d. v.* Prof. Koch *Tuberkelbacillen, etc.*, 1891.

In 1892–93, after the bitter criticism of the followers of Prof. Koch, Middendorp wrote three pamphlets in Dutch, which were translated and appeared in *La France Médicale*, in which he dealt with the evolution of the tubercle, the giant cells, the so-called pure cultures, and the lymph of Koch.*

Having again confirmed the absolute absence of specific bacilli in tubercles still isolated, Middendorp enlarges, in rectifying some inaccuracies in which he thinks Prof. Koch and his followers have fallen, with regard to the structure and evolution of the tubercle. Not even in the caseous and most inward part of the yellow tubercles and their conglomerates did Middendorp detect bacilli; whilst an emulsion of 10 milligrammes of such caseous substance, although deprived of bacilli, produced, when inoculated in dogs, a miliary tuberculosis. He excludes a transmission by means of *invisible spores*, not apt to colour, as well as the supposed *transport* of tubercular bacilli (immobile) through *migratory cellules*.

In one of his letters to me, Prof. Middendorp thus expresses himself:—"The bacilli only accompany pulmonary phthisis in caverns communicating with the bronchia. They are, on one side, immigrants just arrived there; but for the greater part they are the descendants of bacilli which first immigrated into these cavities (which may on this account be compared to sinuous abnormal recesses of the air-passages), and have produced there numerous colonies of various generations. These microbes, therefore, cannot be called *tubercular bacilli*, as they are not found in tubercles as such, and have no etiological relation whatever with tuberculosis. For this reason I maintain that tubercular bacilli, in the sense of Prof. Robert Koch, *do not exist at all.*"

With regard to cultures, Middendorp thinks that their virulence is to be attributed, not to the bacilli in themselves, but to the *necrosing tubercular substance*, in which the bacilli are entangled. Such substance, even free from bacilli, becomes extremely virulent. It goes to form the cultures, and transplants itself in the succes-

* Middendorp, *Tuberkelbacillen Bestaan Niet*, *Open Brief*, etc., Groningen, 1892. *Tuberkelbacillen Bestaan Niet*, *Verdere Bijdragen*, *etc.*, Groningen, 1893. *Es giebt Keine Wharen Tuberkelbacillen*, *etc.*, Groningen, 1894, *La cause (bacillaire?) de la Tuberculose*, *Suite sur la doctrine erronée de R. Koch*, *etc.* (*France Médicale*, 1894, pp. 113—35).

sive transports, which cannot eliminate it or weaken its virulence. It enters as well into the composition of the lymph proclaimed by Prof. Koch for curing tuberculosis; also, its presence can be proved in the cultures from which lymph is extracted.

Consequently (concludes the author) I cannot attach the least value respecting the genesis of tuberculosis, to the inoculations of these cultures, almost pure, because Professor Koch has not inoculated the isolated parasite, but always conjointly with this necrosing tubercular substance, specifically infectious.

So far Middendorp. But his definite conclusions leave, in my humble opinion, two questions unsolved: the first concerning the presence of peculiar bacilli resisting the decolourising agents in sputa of consumption rather than in other sputa; the second question, about their presence even in tubercular nodules of organs not communicating with air-passages (glands, brain, etc.), impugned by Middendorp, but proved by other accredited investigators.

These two points deserve the greatest attention. Even admitting, with Prof. Middendorp, that such bacilli are not at all a species distinct from other forms of *Leptothrix* (and I concur in this opinion), it still remains to be explained why, in the tubercular products, they especially resist the decolourising and are capable of furnishing special cultures. Here is, in my opinion, the real point of the question. I shall speak of this again later on.*

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## The Experiments of Pettenkofer and others on Asiatic Cholera.

The first who tried the ingestion of choleraic matter seems to have been Rochefontaine, who swallowed pills of fæcal matter of cholera patients without any injury to himself. Similar experiments were repeated by Klein at Bombay; afterwards by Wall and others.

During the epidemic at Hamburg in 1892, Prof. Pettenkofer communicated to the Medical Society of Munich Emmerich's and his own experiments with pure cultures of cholera bacilli in broth taken from a quite fresh culture from Hamburg. Of this culture

* Lately the author has given a resume of his observations and views in a newspaper: *La Cause de la Tuberculose*, Paris, Oct., 1895.

Pettenkofer took a cubic centimeter in a solution of bicarbonate of sodium, and suffered only from a slight diarrhœa, with many comma bacilli in the dejecta. Emmerich repeated the experiment in a dietetic excess, and exposed himself to a current of cold air in the night. He suffered from a more acute diarrhœa, with bacilli in the dejecta, and other disorders.

After these experiments, Pettenkofer dared not impugn, absolutely, the pathogenic action of the Comma Bacillus. In a letter to me, he maintains that the key of the etiological problem in the infectious diseases is to be looked for in bacteriology. Therefore, he held the presence of bacilli in the dejecta to be a proof of a certain connection with the choleraic process ; but he did not acknowledge them as the only cause nor as the principal seat of the virus. An epidemic, according to Pettenkofer, is an equation of three incognitæ :—$x$, the specific germ ; $y$, the local conditions ; $z$, the individual predisposition. He attributed chiefly to the want of these extrinsic conditions ($y$ and $z$) the relative harmlessness of the ingathered bacilli, Munich being at that time free from epidemics. In order to confirm that interpretation, it would have been necessary to repeat the experiment in infected countries. Pettenkofer, referring to the experiments of Bouchard,* concluded that the specific virus of cholera *does not arise from the Comma Bacillus*, but is evolved in the *human organism* ; and, once developed, that virus, taken directly from the body of cholera patients, even when deprived of bacilli, transmits cholera to the inoculated animals.†

Frænkel objected that the disorders from which the two experimenters of Munich suffered should have been at once recognised as true attacks of cholera, however slight ; but Pettenkofer had previously requested Bauer and Ziemmsen to verify the nosogra-

* Bouchard, when inoculating substances secreted from the intestines or kidneys of cholera patients, had noticed in the animals under experiment symptoms of cholera ; whilst inoculating on other animals of the same species, simple cultures of the Comma Bacillus, or the product of their material change, had only obtained negative results. (Bouchard, *Les Microbes Pathogènes*, pp. 119—30).

† Pettenkofer, *Ueber Cholera mit Berucksichtigüng d. jungsten Choleræpidemie in Hamburg* (*Münch. Med. Wochenschr.*, 1892, No. 46).

phic table of the indisposition from which they suffered. Emmerich added that the dejecta of the two experimenters, which contained a great number of the comma Bacillus, had been poured in a common closet without any disinfectants, and nevertheless cholera had not broken out at Munich.*

These experiments were, in 1893, followed by those of Stricker at Vienna, who, together with his six pupils, swallowed with impunity large quantities of comma Bacillus. These experiments were, however, impugned by Lœffler.

Lazarus, meanwhile, relates the case of an assistant who contracted a slight cholera infection through having handled many times choleraic cultures.†

Metschnikoff shortly afterwards published new experiments on the subject.‡ Some considerable quantity of a culture nine years old, and harmless to animals, having been swallowed in large quantities, an attack of cholera was produced, which was followed, on the tenth day, by recovery. This experiment was opposed by Drasche; but experienced practitioners proved the case to be a real attack of cholera.§ Metschnikoff, in a second experiment, obtained analogous results from a drop of a recent culture diluted in broth. However, in various other experiments, he could only detect slight disorders. Then he found that *even large quantities of choleraic cultures may be swallowed by man with impunity*, the result of a certain relative impunity being due, in his opinion, to the presence in the intestinal passages of other *antagonistic microbes.*

In September, 1894, whilst Hamburg was free from cholera, Oergel, an assistant at the Institute of Hygiene, in making experiments on guinea pigs, fell a victim to cholera after the seventh day.||

With this exception, all the other infectious cases which happened in laboratories appear to have ended in the recovery of the patient, and did not transmit the disease to other subjects. We

*Frænkel, *Deutsche Med. Wochenschr.*, 1892, No. 48, Emmerich, *ibid.*, No. 50.

†Lazarus, *Berliner Klin. Wochenschr.*, 1893, No. 51.

‡ Metschnikoff, *Annales de l'Institut Pasteur*, May and July, 1893; May and August, 1894.

§ Drasche, *Wiener Med. Wochenschr.*, 1894.

|| *Deutsche Med. Wochenschr.*, 1894, No. 41.

notice also that other observers detected comma bacilli identical with those of cholera in persons who were simply affected by costiveness; that De Crecchio at Naples found them in a case of arsenical poisoning; and others (amongst whom Cunningham) detected, in the dejecta of cholera, ten different bacterial characteristics; whilst, on the other hand, he met with cases of cholera free from any traces of *Spirilla* or Comma Bacillus.

As we see, the facts proving the harmlessness, however accidental and relative this may be, of the cultures and choleraic matter, are neither few nor isolated, and we cannot pass them over, particularly in a matter of such importance. It is difficult to understand how choleraic cultures, ingested in large quantities, may be harmless, whilst a few germs or bacilli introduced, whether mixed with food, drinks, or inhaled, succeed, according to theory, in producing the ravages of cholera.

The question being still *sub judice*, the mind naturally returns to the opinion of Lewis (see the second memoir), who identified the cholera bacilli with the normal comma bacilli of the mouth. It was objected that these cannot be reproduced on artificial soils of culture, whilst the cholera multiply there easily. But we can meet this objection, in my opinion, by saying that these two forms of bacilli represent a single bacterial element, though in two *distinct phases* of existence. In the original phase (buccal comma bacillus) it is a question of *fertilising elements in full activity*, and therefore incapable of multiplying by themselves, though this cannot prevent the same elements, when transplanted into the intestine and afterwards on the cultural media, and precluded from performing their copulative function, from falling back into a retrogressive phase of existence, reverting to simple *vegetative elements*, and so multiplying by fission. In fact, some thought that the cholera bacilli had an affinity to the *Spirilla* and *Spirochæte*, through their aptitude to form articulated filaments, with short bendings.*

For other arguments against the bacterial origin of cholera I refer the reader to the work of Wall.†

* See on this subject the forms of cholera spirilla from broth cultures, in the drawings of Cornil and Babes (*op. cit.*, third edition).

† Wall, *Asiatic Cholera: Its History, Pathology, and Modern Treatment.* Third edition, London, 1893.

## Hypothesis of the Author on the Action of the Bacteria upon the Human and Animal Economy.

Let us examine now whether an eventual simplification of the bacterial species, in the sense that I propose, may be made to agree with laboratory experiments considered within their proper and strict confines.

It is not here the place to take into consideration the action of bacteria in the external world and their destiny in the general plan of nature; as the disintegration of *rocks*, the elaboration of the *humus*, the nitrification and other processes related to vegetal chemistry, etc. Although we have such an array of facts to prove the great importance of Bacteriological Flora, its high destiny in the universal economy, quite different from the supposed morbific action, my task now is simply to consider bacteria in relation to human and animal economy, under three aspects—viz., the healthy state, the morbid state, and in laboratory experiments.

*In the Healthy State.*—Under this first aspect I have elsewhere noticed that the presence of bacteria in such a prodigious number, especially in the mouth and the external genitals, exhibits the character of a real excluding vegetation, destined to protect those natural openings, and to spread itself into other and more distant organs.

The presence of bacteria or their germs in the tissues and fluids of the body had already been an argument for the consideration and researches of many investigators. From a Memoir on *Relapsing Fever* I quoted some passages from Dr. Beale, which are worth repeating :—" The presence of these living organisms in the tissues and fluids of the body is not peculiar to contagious diseases. Bacteria and their germs have been found in numbers, in cases characterised neither by fever, nor by specific symptoms, nor by blood-poisoning, nor by being propagated by contagion. Nay, bacteria grow and multiply, and in all organisms and in all parts of organisms, so very soon after death, either of a part or of the whole body has occurred, as to render it almost certain that their germs must have existed in the organism before death. They live and multiply under many different conditions, and consume healthy as well as morbid matters in a state of decay. In the internal parts of the bodies of man and animals, in their tissues and in their blood, these little germs exist and probably multiply, but very slowly, their life-changes being in abeyance as long as the bioplasm of the body, with its higher life, prevails; but when this succumbs, the bacterium germ bursts its bonds, and its descendants, soon numbering millions of bacteria, assert their sway to the detriment of the part. That bacteria are not in themselves hurtful has been proved most conclusively. Many things we eat contain them in countless multitudes. In the alimentary canal of infants suffering a little derangement of the stomach, bacteria are often present in vast numbers, and in some of these cases not a particle of the contents could be found at any point between the mouth and the anus that was not teeming with bacteria. Many of the secretions may contain them without any perceptible injury to health, while hosts of them are invariably present in the fluids, and in and about the superficial cells of the mucous membrane of the mouth of all persons, even in the most vigorous health." *

To-day the presence of bacteria in the interior of single histological elements is so generally admitted that some wish to argue a kind of *equivocal generation* or derivation of bacteria from the normal granulations of the cellular protoplasm.† So Béchamp thought that bacteria were derived from *microzymas*, or protoplasmic granules, which were apt to *transform themselves* into bacteria as soon as the nutritive activity of the cellule became extinct or suspended.‡ An analogous hypothesis was advanced by Nuesch —viz., that bacteria and ferments were only *secondary cellules* produced by the normal cellules of the vegetal pulp, with this difference, that bacteria would produce themselves when the albumenoids abound in the generating cellules (Zellsaftbläschen); and when, on the other hand, glucose predominates, there would be produced ferments. Sterilisation would only be an effect of the extinction of the protoplasm of the generating cellule, without

* Beale, *op. cit.*, pp. 315—18.

† The equivocal generation of the bacteria has, however, been refuted since 1880 by Dr. Beale (see *How to Work with the Microscope*, London, 1880, p. 205).

‡ Béchamp, *Les Microzymas et les Zymases* (*Arch. de Physiologie*, 1883).

the vitality of which the secondary cellules would be unable to produce themselves.*

Even Prof. de Giovanni observed that, in the disintegration of the red and colourless corpuscles of the blood, of the epithelia and cancerous cellules, they resolved themselves into other and more minute elements, which would constitute the *plastidules* of Haeckel, the colonies of the bioplasts of Altmann, or the bacterial germs.†

Galippe, in three notes (from 1887 to 1891) treated of the presence of micro-organisms in the vegetal healthy tissues; and in a fourth note (1893) described a method of his own for the research of bacteria in normal living tissues, of vegetal or animal origin, and especially in the testicle, liver, and kidneys.‡

The researches of Escherich, Bumm, Mercit, Cohn, Neumann, and Hauigmann revealed the presence of bacteria in the milk of women in perfect health, which occasioned various conjectures about their origin.§

In the third Memoir I recorded the hypothesis of Rud and Arndt—viz., that the moving granules of the salivary corpuscles are but the germs of *Spirochæte* lodging there, in the first phase of their life. In the already recorded Memoir on *Relapsing Fever* I myself described certain groups or heaps of colourless blood corpuscles, from which, by means of the oscillations impressed on the preparation in pressing the thumbs, new *Spirilla* were, now and then, seen to escape.||

These facts (and others which, for brevity's sake, I omit) are a convincing proof of the vast diffusion of bacteria or their germs in our economy, as if their universal presence or ubiquity were co-ordinated to the various metamorphoses and operations of the

* Nuesch, *Arch. des Sciences Phys. et Natu.*, Genève, 1886, No. 10.

† De Giovanni, *Remarks concerning the Inflammatory Process (Gazzetta Med. Veneta*, anno XXIV. e XXV.). Altmann, *Die Elementarorganismen ü. ihre Bezieungen zŭ d. Zellen*, Leipzig, 1890.

‡ Galippe, *Recherches et Notes Originales*, Deuxième Série, 1891, pp. 71—73, Troisième Série, 1893, pp. 37—44.

§ Hauigmann, *Zeitschr. f. Hyg. u. Inf. Krank.*, 1893. See also *Modern Medicine and Bacteriological World*, Oct., 1893, pp. 252—54.

|| Vicentini, *On a Case of Relapsing Fever and on the Discovery of Spirilla in the Blood*, p. 23, and Fig. 2, *h*.

organism. In the second Memoir I touched on the influence of bacteria upon digestion. Now, however, I must appeal to the general law of cellular biology.

Considering the *assimilating* activity of bacteria, on the one hand, and, on the other, their *reducing* activity at the expense of the histological elements in decay, I have often asked myself whether this ubiquity of the bacterial elements is not, perhaps, destined to perform some important service, as, for instance, the expurgation of the fluids to act as scavengers in relation to decayed particles or cells of the body.

As is admitted, the elementary parts or cellules of the single tissues have, each one of them, a fixed cycle of evolution, in which they begin, multiply, and pass away, and other generations take their place. We must therefore presume that the products of decomposition of these extinct particles or *cellulary corpses* with their *residue* become the pabulum of bacteria, in the same way as is daily observed in the epithelia, in the corpuscles of the blood, of the pus, etc. In similar manner the bacteria and their germs, scattered in our bodies, would act as *necrophagous agents*, cleansing the tissues and fluids of the economy, not only of the eliminated substances, but even of the residue of the defunct histological elements, which otherwise would contaminate the body.

The physiological destination in the organism being thus understood, we possess the teleological reason of the presence and ubiquitary circulation of their particles and germs in the various points and recesses of the economy, following the passages and gaps of the blood and the lymph, or migrating slowly from an histological element in decay into another contiguous element. Such particles or germs may be so slender that, even when magnified to 2,000 diameters, they will not be detected *; and can, therefore, cross and pass through the cellular bodies and tissues as through porcelain filters; and more so, for they can penetrate through the intestinal villi and the chyliferous system.

The origin of such circulating particles may be assigned to those internal granules of the common bacterial articulations which are drawn in Fig. 2, *n′*; in Fig. 4, *h*; and in Fig. 5, *e*, *e′*, *f*;

* Beale, *The Microscope in Medicine*, edit. cit., p. 318.

as well as to the young gemmules of reserve enclosed in the filaments, before fully developing into new bacteria. Thus we have, so to say, a fixed capital of bacterial forms, in remarkable or full development, upon the digestive, respiratory, and genito-urinary mucous membranes; and a circulating capital of more rudimentary, slender forms inside the body.

In Disease.—The preceding remarks may apply equally well to the pathological conditions; nay, we may presume that, in local and general affections, the activity of bacteria may become comparatively more intense and agitated. In a vast number of morbid processes we have a great multiplication of histological elements (phlogistic exudations, pus, etc.), and, consequently, a tumultuous succession of new cellular generations, which rapidly retrograde and disappear as the preceding ones. To this prolification and retrogression would ensue, therefore, a larger number of elements and bacterial germs; and likewise a more rapid and agitated multiplication of the same in the diseased parts.

The simple fact that bacteria are present in the products or morbid seats does not imply anything specific, so long as their totally extraneous origin, and their quite independent entity, be satisfactorily proved. Losing sight of this truth, as simple as it is essential, one risks falling into strange exaggerations, and is likely to include the most varied complaints (even the ingrowing nail) in the list of *bacteriological infections.**

In the fever complaints, without proportional local incentive, a similar thing happens. If in these cases the local processes are relatively unimportant, there is, otherwise, an increase not less tumultuous, but even more general, of the cellular metamorphoses, in many provinces or in certain systems of the economy; thence the hypertermia, with a rapid thinning and loss of weight in the body.

This is the reason why, in the acute or chronic disease (for example, tuberculosis), which more severely attack certain systems, the presence of bacteria in the affected parts and other seats is more easily detected. "*Chacun sait*," says Charrin, "*que les mal-*

* On the bacterial origin of the ingrowing nail, see Regnault, *Association française pour l'avancement des Sciences*, Cahen, August 13, 1894.

*adies générales ou locales peuvent faire que nos tissues cessent d'être privés de germes."* *

Now, in my opinion, from this virulent deviation of the primitive activity of the bacterial elements, concurring and multiplying themselves in the affected parts, new properties which bacteria assume would be evolved, often irritating, sometimes polluting, and finally showing themselves through the inoculation of the morbid products or cultures obtained from them in the animals experimented upon. In other words, through the changes brought about, harmless bacteria or saprophytes would develop into infectious or pathogenic bacteria. These contaminating properties then are to be attributed, not to anything original and intrinsic to bacteria, but to an accidental deviation of their activity. And observe that the virulence so acquired would, in its turn, remain still inactive, without any hurtful effect whatever, at least in most cases, if the will of the experimenter did not help to deviate, as far as possible, the natural course of vital phenomena by taking up these bacteria, so modified, in order intentionally to transport them, together with the relative products of decomposition, contrarily to the ordinary course of nature, into the animals experimented upon.

These views largely agree with those of Goldscheider, who believes that bacteria, originally harmless, become infectious or pathogenic through the simple deviation of the primitive biological properties impressed upon them by certain substances (*predisposing substances*). Having once got this deviation, it will be maintained from generation to generation, as the author proves in the *Streptococci* and *Staphylococci.*†

The possible affinity of saprophytic bacteria with pathogenic forms are admitted in a work by one of Prof. Koch's disciples. "The distinction" (so writes K. Fraenkel) "between pathogenic and non-pathogenic bacteria is not so absolute as it at first appears. We know that a fair number of micro-organisms which seem harmless may, in given circumstances, become pathogenic; and we also know, on the other hand, that some pathogenic species

* Charrin, *Compt. Rend. de la Société de Biologie*, 11 Nov., 1893.

†Goldscheider, *Centralblatt f. klin. Med.*, 1893, No. 33.

may lose their pathogenic properties, and form a series of harmless bacteria." And elsewhere, "Considering that all pathogenic bacteria in a period more or less distant were lacking their pathogenic properties; that even the parasitic micro-organisms had originally to lead a saprophytic life; that the pathogenic action depends on a special adaptation to the nutritive conditions, we might consider the loss of this property as a reversion to their old way of existence. It is clear that the tendency of the species to lose the virulence is different, and that some keep it up longer than others. But for all, the virulence constitutes only an accessory, apt to increase or decrease, according to circumstances. Therefore the differences in the virulence cannot assist us in separating, the one from the other, identical species of bacteria."*

I will, on this point, recall an important distinction between *virulence* and *pathogenic* action.

When, in laboratory experiments, we speak of *pathogenic* or non-pathogenic action of bacteria, we mean that we consider an exchange of ideas takes place only as we implicitly hold the action of bacterial cultures in the laboratory to be identical to that of similar bacteria in the genesis of the spontaneous infectious diseases, as exhibited in daily practice, though the truth of this is not quite demonstrated, as the fundamental point of the question has not yet been proved—namely, the supposed aggression of bacteria from without, or by first hand (say, in the pulmonitic or tubercular diseases); nor is it even proved, in the affected part, their *precedence* on the local alteration, as it results from the observations of Spina and Middendorp.

On the contrary, to say *virulent action* is to put the question in its true terms. Thus, we only affirm the fact of certain alterations following upon the inoculation, keeping it distinct from the other fact of the preceding evolution of a spontaneous disease (from the products of which we obtained the culture), and without attributing to this earlier fact what really belongs to the later one, by second-hand—viz., to the irritating or polluting action of the inoculated material. Though mostly unknown, the causes may

* Karl Fraenkel, *Manual of Bacteriology*, Ital. translat., Turin, 1892, pp. 109—123.

be numberless by which a bacterial culture can prove hurtful to animals experimented upon, and often the contamination that follows has nothing in common with the original disease.

We must keep in mind two actual facts in laboratory experiments. The first is that the bacterium inoculated in the laboratory is not a bacterium proceeding from the external world (as is thought to be the case in spontaneous infections), but one purposely taken; and from what source? From a morbid source or from eliminated matter (sputum, pus, fœces, etc.). Now, such a product or material is, after all, but a confusion of various eliminated matters, either in an incipient state of decomposition or quite decayed, destinated to be eliminated by the affected organism, and not inoculated to a sound organism; a material polluted by the cellular decayed bodies, incongruous to life, and even deleterious if taken from the dead body, as is often the case in such experiments.

The second condition of the fact is this :—That a bacterium hardly ever becomes virulent when it is brought back to these sources, for it is originally destined by Nature, let us say, in the mouth; but it may become so when it is introduced into seats not intended–*i.e.*, under the skin, in cavities, or in the circulation, but by Nature, viz. :—The inoculation, as I said elsewhere, introduces at once a huge mass (milliards) of bacterial elements into the internal organs, where Nature did not intend them to be. What wonder, then, that in a great many cases local or general alterations should result? If, on the contrary, you deposit the bacterial culture in the mouth (a habitat destined by Nature for most of these minute beings), it will prove harmless, as is demonstrated by the experiments of Pettenkofer and others in reference to the Cholera bacillus and those of Stagnitta, who for several days ingested rabid matter, without any harm to himself.* The same thing happens with the poison of serpents.

### In Laboratory Experiments.

But it will be objected that the bacteria inoculated in laboratories were, for a certain time and for different generations, nursed in artificial culture media, where it is presumed they may have

* Stagnitta, *Bullettino dell'Acc. Med. di Roma*, 1893, p. 530.

deposited every vestige of those putrid and deleterious substances in which they were previously immersed. And this is partly true. Thus, through frequent transplantations, the virulence of those bacteria may be reduced and even destroyed. Again, the virulence is an acquired property in the affected spots, and may, upon other culture media, diminish or vanish.

But it is understood that such an epuration, *catharsis*, or complete reversion of bacteria to their primitive harmlessness, cannot be immediate; nay, it may perhaps fail altogether. On the other hand, we cannot advocate a purification process of this kind for one property (the acquired virulence) without admitting it implicitly even for the other (the supposed original pathogenic action); as we cannot reasonably expect that, in the artificial cultures, the bacterium should divest itself of the polluting properties acquired in the pathological material (which are a fact) without divesting itself at all of that original, pathogenic efficiency, which is a supposition.

In truth, he who considers the conditions of the artificial cultures must implicitly admit that, if the form, the type, or the supposed specific entity of the bacterium and its supposed pathogenic action is maintained *intact*, in passing from the morbid seat, or from the eliminated matter into the gelatine or broth, this necessarily implies that, in those artificial culture media, are exactly reproduced and maintained the same conditions, bio-physical and bio-chemical, which existed in the product or in the morbid seat. Onè of two things must naturally occur, either the bacterium has degenerated from its primitive condition, or it has not. If it is always the same, it cannot have preserved in quality and quantity the same properties, *but by assimilating the same principles* and casting in the nutritive vehicle the same products of decomposition which first segregated (toxin, toxi-albumen, etc.). The conditions of the bacterium as such then are not substantially changed in the culture; nay, a certain quantity of the impure or decomposed material is transplanted with it; and the same products of decomposition must necessarily be repeated, which, in their turn, concur to produce the pollution, if they do not constitute the principal cause, in the animals experimented upon. If it were not so, the nature of bacterium would not always be the same, or, at most, the bacterium would be thoroughly modified in its biological effects. In successive transplantations an analogous transmission takes place; a certain part of the fermentable mass, together with the relative ferment, is transmitted from one culture to another, as, for instance, happens with leaven or yeast used in baking, which is transported from one parcel of dough to another.

A confirmation of this view can be obtained from the same bio-chemical properties of the ordinary culture media. Let us consider what are broth, gelatine, agar, serum, etc. Are they not organic substances taken from vegetables or animals, living or dead, which were, in both cases, destined to decay and perish after being separated from the living economy? The sterilisation may have transitorily suspended such decomposition, but this decomposition must initiate or recommence, so soon as a fresh corrupted matter, as, for instance, a diseased product, any refuse or cadaverous product, reaches it. In other words, on account of the mixing up or dissemination of the suspected material, the culture medium, although before sterilised, soon becomes decomposed; and from that decomposition no other than virulent and deleterious substances can be produced, to which, more than to the bacterium itself, are to be attributed at last the poisonous effects and the pollution in the animals experimented upon.

I have used the word pollution with good reason, because, in many cases, the disorder caused by inoculations in animals experimented upon resembles a *poisoning* rather than a *disease* in the true sense of this word.

We must in fact make a distinction between the diseases which are *certainly inoculable*, such as cow-pox, syphilis, tuberculosis, etc., from those which are *supposed to be inoculable* (whooping cough, pneumonia, etc.). The first are transmissible through grafting the relative virus, either directly by means of the morbid product in substance, or indirectly through bacterial cultures containing that virus. In these cases it is really the disease which is transmitted, and not an infection of another nature. The bacteria taken from this source, having been in contact with the specific virus, may transmit it from the sick to the healthy, as clothes and linen transmit certain contagions, without constituting, however, the generating principle of the disease.

It is not the same with cases of the second series. Here it is not a question of a *communicated disease* strictly speaking, but of a *produced poisoning*, more or less analogous to the septic or cadaverous infection. This poisoning might partly resemble the original disease from the products of which we got the culture ; if, as often happens, the bacteria, removed from a tissue or an organ (in the patient), will tend to regain (in the mouse or guinea pig) the tissue or the homologous organ. It does not surprise us to find in those organs or tissues of the animal the same bacteria as it would not surprise a farmer (says Zeissl) to see the corn spring up where he sowed it.*

Whether the animal succumbs or survives the experiment, it will be difficult, in my opinion, to establish a perfect identity between this disorder stirred up, mostly tumultuous, and the evolutionary process of a true illness identical with the original disease in man. There are numberless examples of supposed bacilli or pathogenic spores (Letzerich) to prove how often the experimenters are, on that point, led into most serious errors. We may affirm that there is not a single one of them who, with his spores or bacteria, has not pretended to have reproduced the original disease (say whooping-cough), *in all its characters*, confuting one another in turn ; so easy is it to exchange the symptoms of an artificial inoculation with those of a disease *sui generis*, —for example, Septicœmia with hydrophobia (see second Memoir).

In that Memoir we have seen that there is no comparison between the fabulous number of bacterial elements which, at once, introduce themselves in the animals experimented on, and the few homologous elements which, in the spontaneous infections, are supposed to introduce themselves, one by one, from the external world, given and not conceded, that in those spontaneous infections the corresponding bacteria be of external origin, and not simple disseminations of normal bacteria of the economy, as seems more probable to me. In fact, bacteria are most frequently to be found, and therefore considered characteristic of the disease, in the affections of the respiratory and digestive passages. In the diseases really infectious or more distinctly contagious (namely,

* M. von Zeissl, *Sul Diplococco di Neisser*, Ital. trans., Naples, 1887, page 46.

the eruptive fevers and others mentioned by Jaccoud), a true bacterial characteristic has not yet been found.

Here I should stop at the so-called *pure cultures.* Apart from the consideration that we do not even now know whether the bacteria hitherto known are true and complete beings, or simple particles of more complex beings, the difficulties of the isolation of single types, or supposed bacterial species (which confusedly and in huge masses encumber most preparations), are such and so many that a culture can be called pure only in name.*

I have no sufficient data to give a positive opinion upon the specification of *Bact. anthracis*, but fear it is nothing but *B. subtilis* modified either originally or consecutively in the pustule itself.

For many years it has been thought that the successful treatment of Lister should depend purely and simply on the exclusion of bacterial elements suspended in the atmospheric dust, which was reputed to be the only source of the traumatic infections. Now, this opinion is losing ground, and already many surgeons have abandoned the spray, to direct every attention to cleanliness of the hands, of the instruments, and of the medicaments applied.†

Moreover, it is nearly impossible to exclude efficiently the bacteria from the parts operated upon (the same applies to many manipulations in the laboratory), as it has been remarked by Dr. Beale; for, as Galippe says, "*Il n'y a point de barrières pour les microbes.*‡

* "I know nothing more humiliating than to be exposed to difficulties apparently infinitesimal, which always reappear the moment we think we have overcome them. . . . Thus, after knowing the difficulties that the selection of bacteria present, I have become very sceptical about the purity of cultures. . . . The methods boasted of as safe can, in my opinion, give only a relative security."—*Miquel, Etude sur la fermentation ammoniacale, etc. (Annales de Micrographie,* 1891, *p.* 277).

†See on this subject Lawson Tait, *The Pathology and Treatment of Diseases of the Ovaries*, 1883, p. 268, and *British Med. Journ.*, 1882; Bantock, *Medico-Chirurgical Transactions*, Vol. LXIV.; Haegler, *Beitrage zur Klin. Chirurgie*, Bd. 9, 1892; Fritsch. *Bericht ü. d. Ginækolog. Oper.*, 1893; Doderlein, *Deutsche Med. Wochenschrift*, 1893, No. 21, etc.

‡ Galippe, *loc. cit.*, *troisième série*, p. 37. On this subject I recall the remarks of the same author upon a fungus *sui generis (Monilia Sputicola)*, which he saw germinating in filtered and sterilised saliva, attributed by him to germs pre-existing in the filtering pipe *(Journ. de l'Anatomie et de la Physiolog.*, 1885, *Planche xxvii.)*

Bizzozero pointed out, also, the presence of bacteria in the same parts, carefully covered with Listerian dressings.*

"The practice of Lister" (wrote Dr. Beale) "is doubtless excellent, but the data upon which it is based may nevertheless be faulty. It seems very probable that the favourable results of this method of treatment are due, not to the destruction of the bacteria, but to the direct influence of the carbolic acid upon the wound. Carbolic acid interferes with the rapid growth and multiplication of bioplasm, which results after a time in the formation of pus-corpuscles." †

Briefly, the theory of the direct contagium has nowadays supplanted that of the infection through the air-medium. Rather than to germs floating in the atmosphere, the attention is now directed to the organic residues that may be suspended there, or may settle upon and contaminate the instruments, the hands, and the dressing materials (unclean particles, incrustations of pus, exhalations, and excretions). Perhaps the anomalous *bioplasts* of Dr. Beale may have something to do with it.

But even when the traumatic infections should only be due to bacteria, the nature, essentially, and originally pathogenic of them would not at all follow. Microbes totally inoffensive in their *own* natural habitat may become offensive if, either by accident or purposely, they are transplanted in one extraneous to it; therefore, harmless bacteria on the mucous membranes or on the skin, etc., might, in the continuous solutions, in the serous cavities, or in the blood, become corrupting because transplanted to localities neither intended nor consented by Nature that they should occupy.

Concerning the modifications that common bacterial elements may undergo in morbid parts (*e.g.*, tuberculous), it is not difficult to explain, seeing the special bio-chemical and bio-physical conditions which on their arrival they find already established there. The special resistance of tubercular bacilli to the decolourising test and other analogous facts fall in, I think, under this heading. I thought it probable that such bacilli might in part be derived from the same sporules of *Leptothrix* (as they are very susceptible

* Bizzozero, *Man. di Microscopia clinica*, 1st edition, p. 50.

† Beale, *op. cit.*, p. 316.

to aniline colours), fallen in the tubercular seats, and there multiplying in series, under special conditions at present unknown; and partly (those with cancellated appearance) should be derived from other Leptothrical elements still not determined, and modified in an analogous manner. But it is yet to be explained in which way both of them acquired, in the tubercular seats, a special aptitude for retaining the aniline colours.* However, admitting the circulation of common bacterial elements in the internal economy, the presence of the bacilli of Koch would be fully explained, even in the tubercles of the brain, glands, etc., without having to admit their specific origin or their etiological value.

In summing up the reasons hitherto expounded, I venture to conclude—(1) That the pathogenic efficiency of the so-called pathogenic bacteria, in the genesis of the infectious spontaneous diseases (as exhibited by the clinic), is not at all demonstrated; (2) That the laboratory experiments show, nevertheless, a very important fact—namely, certain inquinating properties of some bacterial cultures; (3) that this order of facts, relative to the *artificial infections*, this power of transmissibility of a morbid *quid*, is worth the greatest attention, but cannot, by itself alone, be taken as a distinctive criterion of the bacterial species.

* I refer here to the researches of Prof. Spina on the colourisation and decolourisation of bacteria treated with tannin and other substances. "These researches (he says) contradict the predominant rules in bacteriology. Hitherto it has been said that the Schizomycetes resisting the action of nitric acid constituted a definite species; but the experiments just alluded to show, on the contrary, that this distinctive criterium to characterise the micro-organisms has no foundation, because the action of the same substance, the tannin, renders the more different species resistant to acids." From other experiments not less ingenious, the author concludes that bacteria may find even in altered tissues (as, for instance, in the tubercular seats) such chemical conditions as will fix the tint. *Untersuch. ü. d. Entfärbbarkeit d. m. Anilinf. ting. Bact.* (*Allg. Wr. mediz. Zeitung*, 1887, Nos. 15 and 16). *Bacteriolog. Vers. m. gefarb. Nährsubstanzen* (*Centralblatt f. Bakt. u. Parasitenk.*, 1887, Bd. 2, Nos. 2 and 3).

**Some further Researches on the Morphology of Leptothrix.**

After the publication of the third Italian Memoir, I had occasion to gather some other facts concerning the inferior phases of *Leptothrix*, which will be expounded later on. I shall simply record now a few supplementary remarks upon the already described superior phases.

Together with the most obvious fructifications, with a thin stem, I have drawn, in my last Memoir, other forms of *ears*, less frequent, with a club shaped stem, having two zones of colourisation, etc. (Fig. 27). In these, I was unable to detect the peduncles, sterigmata, or implanted filaments of the sporules in the central stem visible on the other forms. But later on, by a fortuitous chance, I was able to discern distinctly, even in *ears* with a *club*-shaped stem, analogous peduncles, somewhat longer. It occurred in this way:—

At the end of 1892, I had to examine the sputum of a youth affected with an extended *basal pneumonia* of the right side, and in the mass of that sputum I discovered numerous *ears*. I think it was on account of the gravity of his condition and the dryness of the mouth, phenomena having already intervened of *a metastasis in the meninges*, which brought the patient to a rapid end. I kept this sputum for twenty-two days, when, in examining the wet preparation, coloured with gentian violet, using a 1/9 "dry" objective and a condenser of 1·30 N.A., I came across one *ear* with a club-shaped stem, drawn in Fig. 32, *a*, and magnified 820 diameters.

This figure has the characteristic, which I had noticed in other sputa and in the patina dentaria, of being lightly pyriform in appearance. The *glia* (glair) shows a vividly coloured contour; the central stem is also club-shaped, as in Fig. 27; but the club, instead of being cylindrical in shape and of a uniform thickness, exhibits a somewhat conical shape, with its base turned upwards; thence its general pyriform appearance. I noticed that such conical club-shaped ears are, generally, longer than those with cylindrical clubs.

As the colourisation had been too strong, and the preparation was full of numberless stained granulations, I clarified it with a drop of *liquor potassæ* through capillarity (1 : 10), always keeping the ear in the visual field. As soon as the solution of potash reached that field, the coloured granules at once disappeared, and, in spite of the small enlargement, I was agreeably surprised to see, at the same time, the *glia* discoloured, and the thick and very distinct peduncles from the two sides of the central stem, make their appearance, as in Fig. 32, *b*.

In the first place, the dissolving action of potash only acted on the *glia*, the peduncles and the sporules still keeping the stain; but afterwards they also became pale, as I ascertained by using a 1/25-in. objective, although they were discernible, specially by lamp-light. The central stem kept the stain longer, only fading within four hours.

I have already remarked that, even in the *Patina dentaria*, *ears* of the same form are to be found, and upon these as well as upon those with the cylindrical club (Fig. 27) I verified the fact that potash acted in the same way. This re-agent showed me, besides, that the *ears* with club-shaped stem in both varieties are, in the dental patina, more frequent than I had thought. It prevents the drying-up of the specimen, and thus allows one to study its particularities for several days through a residual suffusion of a light azure, which is maintained in the central stem, in the peduncles, and the spores.

After decolouration with potash—viz., after four or five hours—we may obtain another satisfactory effect by letting penetrate, at intervals, under the cover, three drops of the iodurated solution of iodine, which, within another hour, reproduces again the violet colour, though not to the same extent as at first.

With regard to the affinity of the spores in question for aniline colours and their analogy on this point, with the bacilli of Koch, I will give other examples.

I have said in the Second Memoir that, at another time, glycerine may be substituted for water (in the preparations of sputum as in those of the *Patina dentaria*), letting it penetrate through capillarity. I preserved some similar preparations for several months, and was enabled to remark that the common bacteria and the *Pneumococci*, not capsulated, are the first to decolourise (within two or three weeks), under the action of glycerine; the large *Diplo-*

*cocci* (forms of *Sarcina*), coloured in a darker blue tint, hardly decolourise at all. The sporules of *ears* resist longer and only fade within a month or two. Now, the preparations with tubercular sputum, dry, coloured with carbolic fuchsine and treated with nitric acid and alcohol, when they are kept in glycerine, exhibit, likewise, an analogous decolourisation. The Bacilli of Koch gradually fade, and in about a month disappear altogether. The fading is even quicker in the preparations with gentian violet. The bacilli assume there a pale appearance within ten days, and they disappear in two or three weeks. My experiments with dry preparations and deep stainings continued for twelve to twenty hours, and it is to be presumed that the resistance of the bacilli of Koch would result even less in contrary conditions.

Elsewhere, I said that water, when used very warm, if protracted for a few minutes longer, becomes a good decolourising means for the bacilli in question ; but even at the ordinary temperature the water exerts, in the long run, the same action, as it is proved by the dry preparations, kept without balsam, and re-examined now and then, by means of a drop of water, through capillarity. The repeated application of those drops of water is sufficient to see the bacilli become pale and finally disappear.

With regard to the power of resisting the decolourising effects, I remember a case in a preparation of fœcal matter, proceeding from a person believed to be suffering with intestinal tuberculosis. The preparation, stained, by dry process, with carbolic fuchsine, and then decolourised with nitric acid and alcohol, did not exhibit any of the bacilli of Koch ; but in the midst of the mass of other bacterial forms, more or less decolourised, certain colonies or zoöglœic groups of minute oval, pointed cocci were singularly conspicuous ; they were still brightly coloured and girded by slender capsular appearances.

Towards the end of the Second Memoir, I recalled (in a note) the observations of Klein and others on the *forms of ramification* to be found in certain cultures of tubercular bacilli, and on the probable analogy of such bacilli with higher micro-organisms or fungi. Since then various other observers have described new morphological particulars respecting the cultures of tubercular bacilli, especially in regard to the formation of gemmules *(Knospen)* of elements of reserve *(Kernartige Reservestoffe)*, and of protoplasm-granules in the interior of baiclli; in regard to the disposition of vacuoles and knots, analogous to that of filaments of many fungi; to the development of a kind of *mycelium* and even of vertical filaments *(hyphæ)*; and, lastly, upon certain analogies of the tubercular bacillus with the *Actinomyces*. According to these researches, the bacillus of the tuberculosis would not be a Schizomycete, but a mycelium-like fungus; and Belfanti is inclined to classify the tetanus bacillus in the same category. These views clearly, though indirectly, agree with mine in regard to an eventual simplification of the bacterial species; but, for the sake of brevity, I will refer the reader to two articles of Coppen Jones, where previous remarks on this argument are illustrated by new and interesting researches.*

I can say little about staining the ears with other tints. Carbolic fuchsine, with a wet process, vividly attacks the spores, but hardly stains the peduncles. If, a second time, we let a drop of lactic acid penetrate the object, the clods and the ears themselves nearly decolourise altogether; but we get the advantage of using the preparation for a longer time, as the lactic acid keeps it wet for several days. Methyl blue gives a fine transparency to the specimens of the *Patina dentaria* and renders visible a larger number of ears. It attacks the internal stem to a greater extent, but has very little effect upon the peduncles. By substituting glycerine a second time, we obtain a beautiful effect on the ears, having a *wrapping-cap* at the top (Fig. 30), as glycerine promptly decolourises the wrapping-cap, though leaving for some time the internal *ear* coloured. We may speak later on of the advantages of the methyl blue, for the examination of the bundles or matrix of *Leptothrix*. Hæmatoxylin is not suitable for these researches on account of its frequent precipitations.

I would remind those who wish to verify the existence and the details of structure of the fructifications in question that they must

* Coppen Jones, *Ueber einen neuen, bei Tuberkulose häufigen Fadenpilz*. *(Centralblatt f. Bakt. u. Parasitenk.*, Bd. XIII., Nos. 21 and 22). The same author, *Ueber die Morphologie u. Syst. Stellüng des Tuberkelpilzes*, etc. (In the same Journal, Bd. XVII., 1895. Nos. 1 and 2.)

be careful to scrape lightly the *patina dentaria*, avoiding persons with clean teeth and directly after mastication.

Likewise, such fructifications are found even in sputa, and much more frequently than I at first thought, provided that we examine the sputum without previous colourisation, which causes the loss of a great many ears. Those who know them can soon detect them, even in the natural or unstained state, under a simple 1/9th dry objective and a 1·30 N.A. condenser. We can discern, also, their complex mulberry appearance, especially looking for them along the edges of the preparation, where, by their lightness, they are impelled. But the particle of the sputum must be so small as not to pass over the edge of the cover-glass. The *ears* remain intact a long time in the pulmonitic sputum. In one case I detected them on the sixth day, and, in the sputum of the above-mentioned youth, on the twenty-second day after emission. But numerous *ears* can be found even in sputa of bronchitis and in the same tubercular sputa examined in the natural state, and by the wet process. Some were found even in emulsion of sputa with soda or potash during my research for elastic pulmonary fibres, and lately I think I have found also some specimens in fœces.

At a first glance, our *ears* might be taken for certain rarely seen specimens, which somewhat resemble them, as Fig. 33 shows (deeply stained with gentian violet, magnified to 820 diameters). Here it is simply a question of two rows of cocci in mechanical superposition to certain large filaments or bacilli, of opaque appearance and probably old, with some other cocci here and there on the central line, with no trace of proper stem or involving glia. These incidental aggregations of more considerable dimensions (which we might call *false ears)* have nothing, apparently, in common with true fructifications.

In diabetic and albuminous urines, I had already found, in many instances, some small and isolated zoöglœic groups or branches, formed by two or three longitudinal series of bacteria and bacilli, coarsely resembling our *spicæ* or *ears*, as described in the *patina dentaria*. But lately I met, by chance, with a con spicuous and beautiful growth of this kind (resembling a true *zoöglœa ramigera*), in a diabetic urine, with many crystals of uric acid, albumen, and some red blood-corpuscles. This growth, with its tufts, varying from three or five branches to almost a hundred, appeared only two days after the discharge of the urine. The more conspicuous tufts, with their branches grouping around the heaps of uric crystals, occupied the whole visual field of a ⅛-in. object-glass. They were deeply stained by gentian violet, but only slightly by iodine. Their branches—in size and shape resembling, at first, our *ears*, were, however, formed only by two or three longitudinal series of bacteria and bacilli, transversely disposed and sometimes crossing a longitudinal, slightly sheathed chain of short bacilli; but no trace of a central stem nor of peduncles could be seen in their inside. On the third or fourth day, the branches increased more and more in size, with many balls or knots (surpassing in their size the red blood-corpuscles), filled by bacteria, like groups of a small kind of *sarcina*, in a transparent glairy envelope, and other young tufts made their appearance. Lately the older branches intertwined the one with the other, closely resembling the *matrix* of *Leptothrix*, but neither filaments nor stumps were noticed.

I am very sorry to have been (for want of time) unable to give here some drawings of these interesting specimens; but I will treat on this argument, as well as on the *matrix* of *Leptothrix*, on another occasion. I only add that the zoöglœa just described has nothing in common with the *Leuconostoc mesenteroides* of Van Tieghem and Cienkowski, which grows likewise in saccharine media :—(1) on account of its special features; and (2) because I have lately found it also in common urines, in incipient decomposition, and in sediment of sputa emulsioned with potash.*

As for the examination of our ears or grape-bunches, some observers might exclusively rely on the apochromatic object-glasses, I will briefly touch upon the comparative value of such objectives and of the already-described 1/25th-in. achromatic lens for this special research.

My observations were made with a 2-mm. apochromatic glass, cedar-oil immersion, 1·30 N.A. Under this lens and a No. 4 or 6 compensating eye-piece, the grape-bunches, deeply stained by strong gentian violet, appeared, indeed, very conspicuous and

* On the *Leuconostoc mesenteroides*, see the quoted works of Cornil and Babes and Flügge.

brilliant, the sporules, with well distinct outlines, resembling true, though minute, grape-stones, and their disposition on three visible lines being more evident, although their peduncles did not seem so appreciable as under the 1/25th-inch. But, on the contrary, the apochromatic lens proved quite unsuccessful in the examination of bunches slightly stained by the same violet or the nearly uncoloured ones, which could not be perceived without great difficulty. On the other hand, the bunches stained by iodine appeared, *in their entirety*, somewhat distinct, but their internal features did not do so, the sporules being scarcely perceptible and the peduncles not visible at all.

Some satisfactory results were, at last, attained by a combination of a compensating eye-piece (namely, the No. 4, for the Continental tube-length) with the above 1/25th-inch achromatic lens. This combination, affording about 1,000 diameters, gave, however, good results in detecting the peduncles deeply stained by gentian violet, but not so with a pale violet or with iodine.

For illuminating the preparations, in the inclined position of the microscope, I found the arrangement proposed by Carpenter and Griffith, and also recommended by the Rev. Dr. Dallinger in the last edition of Carpenter, very useful—viz., to place the work-table, not in front of the light, but by the side, with the window at your left hand.*

This simple arrangement works so that the specular reflection takes place invariably, under a constant incidence whatever may be the degree of inclination of the tube, with the incident rays reflected by the mirror through a right angle, as in the usual upright position of the microscope. In fact, the tube being parallel to the window, under whatever inclination, it will always be found, with optical axis, perpendicular to the incident pencil of light.†

* Carpenter, *The Microscope and its Revelations*, 3rd edit., London, 1862, pp. 154—155; J. W. Griffith, *An Elementary Text-Book of the Microscope*, London, 1864, pp. 8 and 9; Dallinger, *op. cit.*, p. 361.

†This arrangement would be indispensable in working with a rectangular prism instead of the plane mirror (Beale, *How to Work with the Microscope*, *ed. cit.*, p. 23; Dallinger, *op. cit.*, p. 172), but with the *edge* of the refracting

Then, in order to intercept that part of the same pencil of light which, in such a lateral position of the microscope, would strike the surface of the stage and the preparation, instead of a simple screen, as recommended by Dr. Dallinger, I use a particular shelter or shade, so constructed as to keep thoroughly in the shade not only the stage, but the tube and the surrounding portion of the work-table. This shade is formed by two vertical walls, the one on the left, of black wood, 50 centimetres wide (with an opening, movable and extensible at pleasure, to let in the light on the mirror); the other in front made of black cloth, like a curtain, more or less extensible, having, besides, a sliding roof, also of black wood, through which the eye can be applied to the tube without hindering the hands. Thus we have a sort of dark chamber, diagonally cut, with the most satisfactory effects of illumination.

As in these researches it is a question of preparations mounted by the wet process, the direct lamp-illumination, by turning aside the mirror, would be accomplished by a very low lamp, as in Beale's work,* in order to avoid the vertical position of the stage and the moving of the cover-glass on the slide. With a higher lamp it will be better to use the mirror and the shade, placing the light so as to correspond with the movable opening. In this way we obtain nearly the same effect as in the other arrangement recommended by Dallinger, with the microscope in an upright

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*(Continued from p. 223.)*

angle of the prism perpendicular to the pivot of the mirror, not parallel to it. In the opposite case, a rigidly upright position is required. The same arrangement of microscope with the window at the left hand is very suitable with the concave and especially so with the plane mirror. A mathematical demonstration of the inconveniences of very oblique incidences, based on laws of refraction and reflection, applied to glass mirrors, and of the obstacles occasioned by the interference of direct rays (from the window), together with those reflected by the mirror, would lead us too far. I intend to deal with it in a separate article, giving the explanations kindly obtained from Dr. Dallinger on the subject.

* Beale, *The Microscope in Medicine*, p. 26 and Pl. I., Fig. 1; *How to Work with the Microscope*, p. 352 and Pl. LXXIV., Fig. 3.

position on a large tripod stool, which is placed between the legs of the investigator.*

Here I conclude my first attempt on morphological researches upon the flora of the mouth, and hope that others (more learned in the study of mycetology and algology) may carry them farther, and, if necessary, correct them, advancing them to a degree that my limited private means and daily duty in my medical practice did not allow me to reach.

*Chieti; May*, 1895. THE AUTHOR.

## EXPLANATION OF PLATE I.

### FIG. 1.

*a, a, a, a.*—Particles and granules of myelin in their natural state, × 1000.

*b, b, b, b, b.*—Particles, filaments, and granules of myelin, stained with gentian violet, × 1000.

*c.*—A small mass of myelin in natural state, in the act of extension; *d*, fixed part; *e*, extremity in motion, × 400.

*f.*—Very small vibratile epithelium cell from the air passages, stained with gentian violet, × 2500.

*g.*—Ellipsoidal epithelium cell coated with myelin, natural state, × 400.

*h.*—Salivary corpuscle stained with gentian violet, × 1750.

*i.*—Particles of myelin from the air-cells, Beale, × 2800.

### FIG. 2.

*In Whooping-cough.*

*a.*—Minute bacteria stained with gentian violet, × 800; *b*, the same magnified × 2500.

*In Pulmonitis.*

*b'.*—Small bacillus in formation, methyl violet, × 2500.

*In Whooping-cough.*

*c, c.*—Chains of bacteria stained with gentian violet, × 800.

*d.*—Part of the longer chain, × 2500.

*e.*—Dumb-bell bacteria, some apparently capsular, stained with gentian violet; *f*, the same within a buccal epithelium cell, × 2500.

* Dallinger, *op. cit.*, pp. 345—46, Figs. 291 and 292.

*In Phthisis.*

*c'*, *c'*.—Bent form of bacillus formed by a series of capsular bacteria, stained with gentian violet, × 860.

*In Pulmonitis.*

*f'*.—Two diplococci united in a common apparent capsule, in motion, stained with methyl violet, × 2500.

*In Whooping-cough.*

*g*.—Diplococci bent, and minute bacteria, stained with gentian violet, × 2500.

*h*.—Small pus corpuscle, similar to capsulated pneumococcus, stained with gentian violet, × 2500.

*i*.—Beaded forms of bacteria germinating at one end ; *k*, the same germinating at both ends, stained with gentian violet, × 2500.

*l*.—Diplococci uniting, bent diplococcus, and germinating bacteria, stained with gentian violet, × 2500.

*In Pulmonitis, Pleuritis, and Whooping-cough.*

*l* (below) *l'*, *l'*.—Capsular diplococci, stained with methyl violet, × 2500.

*In Whooping-cough.*

*m*, *m*.—Mature bacilli, stained with gentian violet, × 2500.

*n*, *n*, *n*.—Younger bacilli, stained with gentian violet, × 2500.

*In Pleurisy.*

*n'*.—Bacillus similar to both types *m* and *n*, stained with methyl violet, × 2500.

*In Whooping-cough.*

*o*.—Diplococci and bacteria, rare forms, stained with gentian violet, × 2500.

*p*, *p*.—Large bacteria and diplococci, straight and bent, linked together, stained with gentian violet, × 2500.

*p'*.—The same straight forms of bacteria, but with capsule, stained with methyl violet, × 2500.

*q*.—Bacillus, a single segment, stained with gentian violet, × 2500.

*r*.—Large bacillus, four members, stained with gentian violet, × 2500.

*s*.—Bacteria (minute) and bacilli, beaded forms, morphologically identical with Koch's tubercular bacillus, among minute granules of myelin, *t*, stained with gentian violet, × 2500.

*In Healthy Sputa.*

*u*, *u*.—Bacteria, interwoven, stained with gentian violet, × 600.

*v*.—Beaded form, from which originates a bacillus of the form *n* (on the left, above), stained with gentian violet, × 600.

*v' v'*.—Bacilli, same type as *q* and *x*, stained with gentian violet, × 600.

*x*.—Beaded bacilli, segments of different types, stained with gentian violet, × 2500.

*In Pulmonitis.*

*x′*.—Beaded bacillus, formed of different types, stained with methyl violet, × 1250.

*In Healthy Sputa.*

*y*.—Group of rounded bacteria, stained with gentian violet, × 2500.

*z*.—Chain of bacteria and bacteria of types *q* and *v′*, transformed afterwards into filaments of *Leptothrix*, stained with gentian violet, × 2500.

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FIG. 3.

*a*.—Spore (of *Septosporium* ?) from cistern water, germinating, in boiled sputa, × 400.

*b*, *b*.—Vesicular gemmules of fungus, *d*, *k*, *k′* (*Mucor* ?), stained with gentian violet, × 2500.

*c*.—Single gemmule of above, stained with carmine. (This figure has been tinted violet for purposes of illustration.) × 2500.

*d*.—Portion of a large tuft, from which the gemmules above were detached. *e*, Mother cell of filament. *f*, *g*, *h*, *i*, Filaments of tufts stained with gentian violet. × 800.

*k*.—Fructification of fungus. *k′*, Fructification from which a part of the spores is detached, in glycerine, × 140.

*l*, *l*.—Spores of the above fungus (in glycerine), × 1000.

*m*, *m*.—Spores of fungus, *n*, *o*, *p* (*Aspergillus fumigatus* ?), in glycerine, × 1000.

*n*.—Fructification (meagre) of the same fungus. *o*, More abundant fructification. *p*, Fructification (darker) mounted in glycerine. × 800.

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## EXPLANATION OF PLATE II.

FIG. 4.

*Other forms of Bacteria (Gentian Violet).*

*a*.—Catenula of type *p*, *p* (Fig. 1), incapsulated (*Jodococcus vaginatus* of Miller, ?), from a sputum of Whooping-cough, × 690.

*b*.—Diplococci with and without halo, and small cocci from the *patina dentaria* mixed in the same heap.—*b′*, Very minute diplococcus with halo from the *patina dentaria* mixed with saliva, × 1750.

*c*.—Dumb-bell bacteria, in couples, from the condensed nasal mucus, in normal conditions, × 2500.

*d*.—Diplococci with halo (pneumococci), partly coloured, × 2500.

*e.*—Diplococci without halo, also partly coloured, from pulmonitic sputum, × 2500.

*f.*—Pneumonococci with distinct halos of acuminated shape.—*f′*, Another pale one, without halo, in increase, from a pulmonitic sputum, × 1750.

*g.*—Large diplococcus in a halo, from pulmonitic sputum, × 2500.

*h.*—Diplococci, without halo, in increase, containing internal granules, from pulmonitic sputum, × 2500.

*i.*—Large roundish diplococcus, with halo, surrounded by very minute cocci.—*i′*, Incapsulated catenula, formed of analogical diplococci, from pulmonitic sputum, × 2500.

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FIG. 5.

*Other forms of Bacilli, from a to h, with gentian violet; h′, with solution of iodine.*

*a.*—Bacillus of three types, partly with internal gemmules, partly clear, partly opaque, from sputum of *influenza*, × 1250.

*b.*—Bacillus, partly opaque, partly clear, from saliva, × 1250.

*c.*—Bacillus of four types, from saliva, × 1250.

*d.*—Bacillus partially coloured, containing a transverse dumb-bell bacterium (*Bacillus buccalis maximus* of Miller, ?), from *patina dentaria*, × 2500.

*e.*—Spindle-like bacillus, internally granulous, from sputum of bronchitis.—*e′*, Analogical bacilli, but cylindrical, from pulmonitic sputum, × 1750.

*f.*—Two very slender bacilli, one mono-articulated, the other granulous, from pulmonitic sputum, × 2500.

*g.*—Very slender bacillus with three knots, from the same sputum, × 2500.

*h.*—Comma bacillus (*Spirillum sputigenum* of Miller) from pulmonitic sputum.—*h′*, Serpentine bacillus, shaped like a spermatozoid (coloured with solution of iodine), from the *patina dentaria*, × 2500.

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FIG. 6.

*Tufts of Leptothrix, in normal condition, from* Bizzozero, × 400.

*a.*—Bundles of filaments of Leptothrix.—*b*, Articulated filaments. *c*, Spirilla from *patina dentaria.*

*d.*—Tuft of Leptothrix from a concretion of the lachrymal bag.

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FIG. 7.

*Jodococcus vaginatus*, Miller, × 1100.

FIG. 8.

Tuft, in natural state, of *Leptothrix gigantea* of a sheep, from Miller, × 540.

*Sp.*, Spirillic filaments. *V.*, Vibrio-filaments.

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FIG. 9.

*Roots, Swellings, and a few contracted fertile Filaments of Leptothrix, from the patina dentaria*, × 850.

*a.*—Fragment of fertile filament, with two roots, in natural state (*haustoria*, ?).

*b.*—Fertile filament with three roots, and a swelling at the apex, in natural state.

*c.*—Various forms of swelling at the apex, or small heads of young filaments, with gentian violet.

*d.*—A large, somewhat woody filament, ending in a small chain (*Leptothrix buccalis maxima* of Miller), with a contraction, from which springs forth, at last, a slenderer fertile filament, with gentian violet.

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FIG. 10.

*Three fructifications by Ears, isolated, with gentian violet, from the patina dentaria*, × 1250.

*a, a.*—Gemmules of reserve, within the stalk.—*b*, Intact sporules.—*b'*, Residual sporules on the point of dropping from the *ear*.

*c.*—*Ear* grafted upon an older stalk, with knots.

*d.*—*Ear* grafted upon a younger stalk, also with knots.

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FIG. 11.

Tuft of Ears seen from above, with weak methyl violet, from *patina dentaria*, × 1250.

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FIG. 12.

Tuft of Ears seen in profile with weak fuchsine, from *patina dentaria*, × 850.

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FIG. 13.

Two Ears jutting out from a tiny island of *materia alba*, with unacidulated solution of iodine, × 1250.

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FIG. 14.

Productions by *points* (pseudo inflorescences), in natural state, from *patina dentaria*.

*a.*—Branched pseudo-inflorescence, bearing small spindle-like bacilli (male organs, ?), × 690.

*b.*—Isolated pseudo inflorescence, bearing analogical bacilli, × 690.

*c.*—Fragment of pseudo inflorescence, bearing spindle-like and comma bacilli together (male organs, ?), × 690.

*d.*—A dropped spindle-like bacillus, more enlarged, in quick motion (*Bacillus tremulus* of Rappin), × 1250.

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Fig. 15.

Articulations and comma bacilli, with vacuoles, in apparent conjugation, with unacidulated solution of iodine, from *patina dentaria*, × 2500.

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Fig. 16.

Specimen of fructification by *ears*, filling up two visual fields (coloured, for two hours, with gentian violet) from a very slender layer of pulmonitic sputum, on the seventh day after the emission, × 850 and × 2500.

*A.*—A principal tuft. *B.*—A part of it drawn down to the bottom from the pressure of the cover-glass, × 850. (This specimen is erroneously marked Fig. 14 in the Plate.)

*a.*—Denudated Stalk, containing gemmules of reserve, × 850.—*a'*, A part of it more enlarged, after a long saturation with glycerine, with a trace of knot, × 2500.

*b.*—A long *ear*, mostly scattered, × 850.

*c.*—A broken ear, with clear section, × 850.

*d*, *e.*—Cumuli of sporules dropped from the adjacent *ears*, × 850.

*f*, *g.*—Points of two *ears* bent down from the pressure of the cover-glass, × 850.

N.B.—Other fragments of *ears* were seen scattered about the visual fields, around the specimen here delineated.

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## EXPLANATION OF PLATE III.

Figures taken from Billet :

Fig. 17.—Articulations analogous to the pneumococci, belonging to various bacteriological species on the point of passing to the *dissociated* or to the *zoöglœic* state. *a*, A fragment of six articulations of *Cladothrix dichotoma* in disintegration, × 320. *b*, Articulations of the same *alga* in more advanced disintegration, × 1600. *c*, *d*, *Bacterium osteophilum*, ready to pass to the zoöglœic state, × 600. *e*, A fragment of four articulations of *Bacterium osteophilum* on the point of passing to the scorpioidal state, × 600. *f*, Small zoöglœic group of *Leptothrix parasitica*, Kützing, × 745.

Fig. 18.—Other analogous articulations. *a*, Rectilineal forms of *Cladothrix dichotoma* in the act of passing to the zoöglœic state (stained first with solution of iodine, then with methyl violet or fuchsine), × 600. *b*, Beginning of the zoöglœic state of the *Bacterium osteophilum* (colouring with vesuvine, then with methyl-violet, and lastly with solution of iodine), × 600. *c*, Beginning of its scorpioidal state (same colouring), × 600. *d*, Same as *b*, enlarged, × 1600. *e*, Zoöglœic group of *Leptothrix parasitica*, Kützing (colouring as in *a*), × 320.

,, 19.—Ramified zoöglœa of *Cladothrix dichotoma*, formed by 21 branches, upon a single peduncle (same colouring as Fig. 2, *a*), × 120.

,, 20.—Superior endings, more enlarged, of two of the preceding branches (same colouring). *a*, *b*, Tops of branches, × 600. *c*, A portion of one of them, more enlarged, containing bacteria and bacilli of different shape and form, × 1050.

,, 21.—*Leptothrix parasitica*, Kützing, vegetating on two crossed stems: the one of *Zygnema*, the other of *Cladothrix* (colouring with vesuvine and methyl-violet, then with solution of iodine), × 600. *a*, *a*, Stem of *Zygnema*. *b*, *b*, Stem of *Cladothrix*. *c*, *c*, *c*, Filaments of *Leptothrix parasitica*. *s*, *s*, Their bulbs or engrafting spores.

,, 22.—A part of the previous figure, more enlarged (same colouring), × 1600. *c*, *c*, *c*, Filaments of *Leptothrix parasitica*. *s*, *s*, Their bulbs or engrafting spores.

ORIGINAL FIGURES:

,, 23.—Ears or clusters of *Leptothrix racemosa*, with acidulated solution of iodine. *a*, Young cluster, with small, thin spores upon their sterigmas, × 1700. *b*, *b*, Groups of bacteria and comma bacilli. *c*, A matured cluster, with sterigms and thicker spores, × 1170. *d*, Fragment of ear, with sterigmas without spores, excepting two spores, still adhering, × 1170.

,, 24.—Cluster showing the peduncles or sterigmas, funnel-shaped, and the central stem, containing minute gemmules of reserve, stained with weak gentian violet, × 3100.

,, 25.—Ear, foreshortened, seen upon a turned-up stem, with a terminal rosette of six sterigmas and relative spores on the top, stained with acidulated solution of iodine, × 1700.

,, 26.—Clavated stems, stained with gentian violet, × 1700. *a*, Stem with a perfect clava, but not yet fructified. *b*, Stem with a short clava, in formation.

,, 27.—Ear with two colouring zones, with clavated stem (fructified) and more compact spores (teleutospores ?), stained with gentian violet, × 1700.

Fig. 28.—Sheath expansion (young antheridium or spermogonium ?), containing slender transversal fine beams (future fecundating elements ?), stained with strong gentian violet, × 3100.

,, 29.—Ear doubled up at the top, stained with acidulated solution of iodine, × 1700. *a*, Long stem. *b*, Point of the ear, doubled up obliquely and pressed down on the middle third. *c*, Elbow simulating a gibbosity.

,, 30.—Ear, apparently pyriform, through superposition of adventitious cocci and bacteria, stained with gentian violet, × 1170. *a*, Cap, at first stage of colourising, pale, formed by adventitious cocci and bacteria (through contact of lips ?) up to the middle, *a'*, *a'*, of the internal ear. *b*, *b*, Internal ear, brilliantly coloured. *c*, Stem.

## EXPLANATION OF PLATE IV.

Fig. 31.—*Schmelzoberhautchen mit Leptothrixartigen Wucherungen* (Micro-organisms from the Enamel cuticle), from a microphotograph sent by Prof. Miller to the author, × 400.

,, 32.—*a*, "Ear" with conical club-shaped stem, with two zones of gentian violet staining, with peduncles quite invisible. *b*, The same *ear* after treatment with potash, showing very distinct peduncles, × 820.

,, 33.—Rows of cocci fortuitously adhering to old bacilli *(false ears)*, with some cocci in the central line stained gentian violet, × 820.

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Finis.

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