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Bacteria, Their Relation to Modern Medicine, the Arts and Industries

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BACTERIA, THEIR RELATION TO MODERN MEDICINE, THE ARTS AND INDUSTRIES.

BY L. H. PAMMEL.

It has been customary for the president, in making his retiring address, to choose some popular subject and discuss it on broad lines. In some cases my predecessors have given a resume of the scientific literature in our own State, and I need not say that we all feel proud of the work accomplished by this small band of workers. I shall venture, in this address, to discuss the subject of Bacteria along general lines and hope I may be able to correct some popular misapprehensions concerning the subject.

The word Bacteria has almost become synonymous in the minds of some with certain diseases in lower animals and man, but this popular construction is so erroneous, that I propose in this address to show the extent and importance of the question of bacteriology to many important problems.

We shall treat this question in the following way: History, methods of study, structure, question of species, hygienic problems, Bacteria and their relation to economic problems in agriculture and other industries.

We are told in an admirable treatise by Loeffler[2] on the historical development of bacteria that the presbyter, Kircher more than 235 years ago observed that air, water, soil, cheese and putrefactive substances contained countless numbers of "worms" as he designated them. Having observed these living organisms he at once concluded that the Italian plague of 1656 could be traced to these "worms;" but, the most remarkable of the early workers was Antony von Leeuwenhoek a mechanic of Delft, Holland, who had learned the art of making lenses while an apprentice in a linen factory. With his simple lenses and excellent powers of observation he was enabled to observe Bacteria of putrid material, tartar of teeth, etc. Some of the forms were figured and described. He says[2]: "Mit grosser Bewunderung sah ich, dass uberall in den genannten Material viele sehr winzige Thierchen enthalten waren, welche sich auf die ergoetzlichste Weise bewegten." From his figures and descriptions one cannot doubt but that he was dealing with bacteria. We cannot here give in detail the conclusions reached by Vallisneri, Goiffon, Nicholas Andry and Varro, but they concluded that these organisms caused disease. The celebrated Linnaeus[3] could not dispel from his mind that certain living organisms caused disease. The learned Viennese physician, Marcus Antonius Plenciz, discussed in a clear and logical way the cause of contagious diseases. He

[1]Vorlesungen ueber die geschichtliche entwickelung der Lehre von den Bacterien fur Aerzte und Studirende, Erster Theil bis zum Jahre 1878, 37 figures and 3 plates pp 252, Leipzig, F. C. W. Vogel, 1887.

[2]Loeffler l. c. p. 5.

[3]Loeffler l. c. p. 8.

argued that a period of incubation must occur for each disease, and as wheat seed only produces wheat, so too the particular seminia of a disease only produces that disease. He too argued that decomposition is brought about when sown with material, that this material propagates and grows.

In 1820 Ozanam wrote a learned dissertation on epidemic and epizootic diseases, in which he says it is not neceesary to show that the theories of Plenciz and others, are purely hypothetical and erroneous. Notwithstanding that Ozanam doubted the correctness of the view of Plenciz on the contagious nature of diseases, others continued to carefully study the organisms of water, etc. Russworm designated them by their form, and, although he carefully studied them, this subject advanced but little. A host of scientists studied these so-called animals because it afforded great amusement. Little, however, was added till the celebrated Danish investigator, Otto Friederich Mueller, of Copenhagen, in 1776, made an exhaustive study of these so called Infusoria. He recognized the great difficulty in the study of these organisms, for he says "the certain and clear distinction of these requires so much time and sharp discrimination with the eyes, as well as excellent judgment and so much evenness of mind and patience that scarcely anything else equals it." He described ten species of the genus Monas and thirty-one of the genus Vibrio. He used such characters as motion, biological characteristics, Morphology and habitat. These germs were accurately figured so that it has been possible to recognize some of the species. But we must rapidly pass in review the work of Paula Schrank, who divided these vibriones into those with motion and motionless. Bory de St. Vincent placed these low forms in the family Vibrionides, deriving some of his characters from Auguillula. He recognized five genera.

SPONTANEOUS GENERATION.

The theory of spontaneous generation long held sway in the popular mind. During the middle ot the eighteenth century the defenders of this theory promulgated their doctrines and for a long time the field was held undisputed. In this cause were enlisted such men as Needham, who observed the development of living organisms from grains of wheat and barley.

Although this material had been boiled and heated for a time and the vessel closed, still they developed. The arguments seemed impregnable and Buffon, and Wrisberg, Treviranus and others during the early decades of this animated discussion championed the cause of abiogenesis with a great deal of emphasis. During the fifties and sixties and in the seventies and even down in the eighties abiogenesis has had its defenders in such men as Pouchet, Joly, Musset, Wyman, Mantegazza, Huizinga, Bastian, and Wigand[4]. It is strange that the clear and logical thinker Wigand should, as late as 1884, assert that bacteria can arise independently without pre-existing forms from an organic substance *e. g.* spontaneously.

This, it seems to me, shows a lack of the proper methods of experimentation. Wigand had scarcely reached the stage of experimental work, where it was left by Spallanzani in 1769, who, as some one has said, was the most celebrated experimenter of that century. We may also mention Bonnet who was in thorough accord with this celebrated exper menter. With Spallanzani began the system of sterilization and making of tests to set aside spontaneous generation. It led Appert to utilize heat in the preservation of organic substances and has opened the way for glorious modern achievements.

Later investigators held that Spallanzani's experiments were not above criticism,

[4] Entstehung und Fermentwirkung der Bakterien vorlaufige Mittheilung. Zweite Auflage, N. G. Elwertssche Verlagsbuchhadulung, 1884, pp. 40 Seep. 5.

but they were fortified and strengthened by Schwann, who demonstrated that fermentation could not occur unless germs were present. The presence of these organisms was not denied. Braconnet (1831), Berzelius (1827) and Liebig, that brilliant, but conservative chemist, strongly held that these ferments simply accompanied the process of fermentation. Some held that the action of these ferments was entirely catalytic. Schwann, however, showed that various substances heated sufficiently under ordinary conditions will decompose, but if the air before having had access is heated, putrifaction did not occur. Schroeder and von Dusch were able to show that these precautions are not necessary, since a cotton plug will completely filter out all germs, and owing to this, which now seems a small matter, bacteriology has accomplished wonders in modern medicine and the arts. Hoffmann, Chevreul and Pasteur demonstrated that cotton is not essential for holding out germs. This can be done by simply drawing a tube out and bending it. As the germs simply follow the law of gravity they cannot enter.

In rapid succession the work of Pasteur, Klebs, Lister, Rindfleisch, Burden-Sanderson set at rest the theories of spontaneous generation. They are no longer advocated. All existing bacteria arise from pre-existing forms; so much is settled.

Bacteria are, no doubt, subject to the same general laws as to the origin of species as other living beings are; their growth and reproduction is determined in a measure by surrounding conditions. Bacteria are, no doubt, modified by climate and environment as are other living plants, but as yet we know little about this.

We may now ask, what are bacteria? Undoubtedly, plants amongthe lowest in the vegetable kingdom. In form, method of growth, and reproduction, they strongly resemble *Schizophyceæ*. Chlorophyll is absent. A few of the species described by Engelmann[5] and Van Tieghami[6] like *Bacillus chlorinum* and *B. virens* have Chlorophyll, and hence assimilate, but it may be doubted whether these forms are bacteria. They are, no doubt, closely related, and are important links in the chain of evidence showing the relation of bacteria to some of the algæ. With these exceptions they are fungi which do not form true hyphæ, nor do they make a true apical growth or branch; pseudobranching occurs in forms like Cladothrix. In shape bacteria are round, elliptical, rod-like comma, and spiral, sometimes growing in threads, and now and then certain aberrant forms. A peculiar group is found in Dr. Thaxter's[7] Myxobacteriaceae, which resemble Myxomycetes. "These consist of motile, rod-like organisms, multiplying by fission, secreting a gelatinous base and forming pseudoplasmodium-like aggregations before passing into a highly developed cyst-producing resting state, in which the rods may become encysted in groups without modification or may be converted into spore masses." Bacteria reproduce by division, the cell divides and two new individuals are formed. Many species form spores; these are usually of the endogenous character; a few form arthrospores, as in *Leuconostoc* and *Cladothrix* In this genus we have the curious anomoly that *C. intricata*, Russell, branches like *Cladothrix* and forms endospores like *Bacillus*[8].

The cells are all provided with a cell-wall which appears to be made up of cellulose. Many of the species have motion, and this is in all cases probably due to

[5] Bot. Zeitung, 1882, p. 321.

[6] See Fluegge Mikroorganisms, p. 289; DeBary Bacteria. p. 4.

[7] On the Myxobacteriaceæ, a new order of Schizomycetes, Contributions from the Cryptogamic Laboratory of Harvard University, XVIII. Bot. Gazette, vol. XVII., pp. 389, 406, with plates XXII.-XXV.

[8] Zeitschrift fur Hygiene Vol. XI., 1891, p. 192.

cilia which may be numerous, coming from the periphery, as in Typhoid fever Bacillus, or several from the end, or a single one at one end of the extremities. Motion has recently been observed in a *Micrococus*. In some cases the cell-wall is extensible, some species are provided with a gelatinous envelope, the thickness and composition varies in different species. In some this sheath is a carbohydrate nearly like cellulose or in some putrefactive species, it is an albuminoid known as mycoprotein. In some cases these sheaths contain iron, other colors are sometimes found in the sheath, blue, yellow, red, etc., but it may be questioned whether these colors in all cases really belong to the sheath, although they do in some cases.

The contents consist of protoplasm which in some cases appears to be nearly homogeneous, but in a number it contains albuminoid bodies. In *Beggiatoa roseo persicina* it is colored according to Lankester[8a]. In *Clostridium butyricun* small refrigent granules occur that color blue on the application of iodine, in that respect they are similar to the granulose of starch. *Beggiatoa alba* and others contain highly refrigent granules of sulphur which are readily made out. Nucleus occurs as Buetschli[9] and others have shown. These authors believe that the nucleus is large. Minot[10] says, "This important discovery in conjunction with the extraordinary power of proliferation in bacteria confirms our generalization that a small proportion of protoplasm is essential to rapid growth." Koch, however, holds that the nucleus is not distinctly separated from the remainder of the protoplasmic mass.

Bacteria are among the smallest of plants, they vary in size from 0.0001 millimeter (Iu) or less, to 0.004 (*Bacillus crassus*) in width, length varies greatly. Bacteria are ubiqutous occurring in soil, air, water, ice, snow, dust, animals' plants. They are especially common in filthy and putrid substances; their use in such places is so important that we shall discuss this at greater length in the proper place.

SYSTEMATIC POSITION.

It will be seen from what has previously been said that the earliest investigators variously arranged bacteria. It seemed certain to them, that they were animals, for had they not motion? The learned Ehrenberg in 1838 ascribed to some, complicated digestive organs, owing to the way in which coloring matter was taken up. He recognized the division Monadina and Vibrionia.

We may now mention another systematist who still adhered to the animal nature theory: Felix Dujardin in his "Historre naturelle des Zoophytes," 1841, admirably figured the species in some cases, and it is worthy of note that this man observed that these "Infusoria" brought about certain chemical changes. He found that oxalate of ammonia which had been added to his culture material entirely disappeared when the germs had been growing in it for a time.

Perty, in 1852, indicated that some of these so-called animals were plants. Two years later Cohn published an admirable paper on the microscopic algae and plants in which he clearly indicated that the organisms in question were plants and not animals. Nægeli had previously recognized that some of the colorless forms found on algae were fungi, they did not assimilate like algae. In 1857 he brought all these forms together and called them Schizomycetes, a term generally adopted by bacteriologists at the present time.

8a Quart. Jour. Mic. Science, Vol. XIII, 1873, Vol. XVI.
9 Ueber den Bau der Bacterien, 1890.
10 Proc. American Association Adv. of Science, Indianopolis meeting, 1890, p. 284.

We may now briefly discuss the later classifications of bacteriologists. These begin with Davaine in 1868, who placed them in the following genera. *Bacterium, Vibrio, Bacteridium* and *Spirillum.* Hoffmann (1869) also adopted form as a leading character. He lays stress upon the fact that motility is not a good character, that this character may be absent or present depending somewhat on the conditions of the medium and temperature. As he was not dealing with pure cultures his observations in this respect are of little value. Ferdinand Cohn[11], of Breslau, who devoted himself largely to a study of bacteria since 1853 formulated and adopted an excellent system of classification which was largely followed, till better methods of culture were in vogue. Cohn's work made a profound impression on the chaotic condition of the science at that time. Cohn was too able an investigator to rely exclusively on the morophology of these organisms, for he states that germs cannot be separated morphologically, since they will show different chemical and physiological characters. He was not able to use many of these characters, since culture methods were very crude at that time. How far his predictions have held is only too well known to workers in this field at the present time. He made three groups—Chromogenic, Zymogenic and Pathogenic, characters which certainly find use in our present systems of classifications. Cohn believed that species of bacteria could be established just as well in this group of plants as in more highly developed organisms. The views of Cohn were not left unchallenged, for in 1874 Billroth published his researches on *Coccobacteria septica*, an organism which he obtained from milk serum. He argued that in different media the same species varies greatly; he says "es gibt bis jetzt Keinerlei morophologische Kennzeichen irgend einer Micrococcus-oder Bacterien form, aus welcher man schliessen konnte, das-sie sich nur bei dieser oder bei jener Krankheit in oder am lebenden Korper entwickeln konnte." Lister, who has achieved such renown because of the introduction of antiseptic treatment of wounds, believed that morphological characters used by systematists from Ehrenberg's to Cohn's time were not to be relied upon, because he thought species changed in different media. Thus he held that his *Bacterium lactis* when grown in decoction of beets, urine and other media presented quite different morphological characters. In some media it had motion, in some it had not. He overlooked the fact that this single drop of milk contained many organisms. The accomplished bacteriologist, Buchner, at a much later day, thought *Bacillus subtilis*, a harmless species, could be converted under different conditions into *Bacillus anthracis*, a virulent pathogenic germ. Dealing with such small objects and methods of culture in vogue at that time caused a mixture of the two species. Is it to be wondered at that mistakes should have been made and wrong conclusions drawn?

We may conveniently now refer to the work of Hallier, a German botanist, who became greatly impressed with the work of DeBary and Tulasne on the polymorphism of higher fungi. Why should not this polymorphism occur in these small organisms? Luders had indeed advanced the theory that they were connected with higher fungi. Hallier constructed a culture apparatus in which his isolated germs were grown. Moulds of all kinds appeared, and the same common moulds appeared in widely different cultures. He concluded that the medium is the most important element in showing this polymorphism. He states that it is nonsencical to describe separate species of yeasts and bacteria with long names. His study of Asiatic cholera, diphtheria, glanders and other contagious diseases convinced him that they had their origin in a Micrococcus

[11]Untersuchung ueber Bacterien, Beitraege zur Biologie der Prflanzan, Vol. I., 1877, p. 127.

which was derived from higher fungi or algae. Many physicians and scientists were inclined to accept these wild doctrines. Was not the evidence good? Had he not the microscopical and culture demonstrations? Opposing these theories were two eminent botanists, DeBary and Hoffmann, the latter a strong believer in polymorphism, both were able investigators, the former one of the most brilliant botanists of our time. They held that species of bacteria could not be changed into higher fungi.

DeBary maintained that the first canon had not been observed, namely, watching the development of these forms. Hoffmann went so far as to state that polymorphism does not occur in bacteria. But we cannot close this part of the subject without referring to the work of Nægeli, an eminent German botanist and author of a celebrated work on "Die Niederen Pilze," who maintained that species of *Schizomycetes* cannot be defined by morphological characters.

DETERMINATION OF SPECIES.

For several years the writer has been studying the flora of butter, cheese, milk and cream. Many species have been found, and of these but few could be located, largely due to the inperfect descriptions; the chromogenes were much easier because more attention has been given to them and their color affords good characters. Saccardo[11] in his Sylloge Fungorum, gives descriptions of a large number of species. This work of De Toni is, of course, largely a compilation, the descriptions are largely abbreviated so that it is a hopeless task to properly or even approach the species. The tables of Eisenberg[12] are much more satisfactory, though even these are sometimes wanting in fullness. Nevertheless Eisenberg's tables are samples of what should be done in this line of study. The works of Flugge[13], Sims, Woodhead[14], Crookshank[15], will enable one to locate some of the more common species. Of special importance in this connection I may mention the paper by Edwin O. Jordan[15a] on the Bacteria of Sewage. The descriptions of the species found there are especially full. The paper by Welz[16] on the bacteriological examination of air, contains excellent descriptions of several species. A paper by Dr. H. L. Russell[17], on the bacteria occurring in the water of the Bay of Naples, is certainly a model in its way.

The imperfect descriptions of pathogenic organisms are not so numerous because of the importance of the subject from a hygienic standpoint. In some cases these contain many valuable notes on the biology of the organisms, as Kruse and Pansini[18] on the Diplococcus of Pneumonia and related Streptococci. The excellent papers of Dr. Theobald Smith[19] that are replete with biological and

11 Sylloge Fungorum, vol. VIII, pp. 923-1087.

12 Bakteriologische Diagnostik Hilfstabellen zum praktischen Arbeiten, second edition, pp. 159. Leopold Voss, 1888.

13 Die Mikroorganismen, mit besonderer Beruecksichtigung der Aetiologie der Infections Krankheiten, pp. 692, with 144 figures. Second edition, Vogel, Leipzig, 1886.

14 Bacteria and their products, pp. 459, with 20 photo-micrographs, London, Walter Scott, 1892.

15 Manual of Bacteriology.

15a A report on certain species of bacteria observed in Sewage. Mass. State Board of Health, 1890, Pt. II, p. 821.

16 Bacteriologische Untersuchungen der Luft in Freiburg und die Umgebung. Zeitschrift fur Hygieine und Infectionskrankheiten. Vol. XI, p. 121.

17 Unterschungen ueber im Golf von Neapel lebende Bacterien. Zeitschrift fur Hygiene und Infectionskrankheiten. Vol. XI, pp. 165-206, plates XII-XIII and three figures.

18 Unterschungen ueber den Diplococcus pneumoniae and verwandte Streptokokken. Zeitschrift fur Hygiene und Infectionskrankheiten. vol XI, p. 227.

19 Hog Cholera Report, 1889. Swine Plague Report, 1891, U. S. Dept. of Agrl., etc.

physiological observations. Dr. Sternberg[20] has also recorded a very large number of observations on the bacteria found in connection with yellow fever.

One of the most important works of its kind ever issued is Sternberg's[21] Manual of Bacteriology. This should be in the hands of every bacteriologist. The descriptions are so thorough that little more need be desired. Most of the species are easily identified by the diagnostic found at the end of the volume, while the descriptions are very thorough and complete.

CHARACTERS IN BACTERIA.

It is convenient here to discuss what characters should be used in the description of bacteria.

Those who have given any attention to the classification of *Schizomycetes* are aware that the work of purely systematic botanists like Winter[22], and Burrill's[23] translation of the same; Trevisan[24], DeToni, Cohn cannot be used or offer, in sufcient data, since morphological characters to separate species are not reliable. Many species are of the same size and shape. The species, however, seem to be quite constant in their morphological characters as shape and size do not appear to vary much within a species. Cohn[25] largely used shape and color in the determination of species, but this was largely pioneer work and many of the species defined by him cannot be recognized, and this is worse as we go back in the history of this science. Zopf[26] has been an earnest advocate of pleomorphism of species, and his classification rests on this doctrine. But pleomorphism is not so general as was at first supposed by Zopf. It is true that some species produce resting spores that resemble cocci as in Anthrax and other bacilli, but they never vegetate as such. But pleomorphism does exist in certain forms as in the group to which *Cladothrix*, *Beggiatoa*, and *Crenothrix* belong. These are truly pleomorphic, at least if we are to trust the work of those who have given the subject attention. In some forms, culture experiments have shown that a certain amount of pleomorphism does exist as in *Cladothrix intricata*, Russell. But in many cases the facts of pleomorphism have not been brought forth by culture experiments as was at first supposed. Frænkel[27] makes the statement that these organisms (*Cladothrix*, *Beggiatoa*) do not belong to bacteria, although they may be closely allied to them. "We may therefore maintain that, thus far at least a many formed species of bacteria has not been observed, and the rule one can distinguish by the growth and from clearly recognizable genera and species of bacteria, which do not run into each other."

Morphologically then, the different species are distinct, quite constant, although many species are similar. Our main reliance must be on physiological characters. And this is used nowhere else in the vegetable kingdom. Physiological characters are sometimes used in the classification of animals, as in the *Hexacorallinna*. The *Madroporiæ* secrete stony skeletons while the *Actinariæ* do not. In other

[20]Report on Etiology and Prevention of Yellow Fever, U. S. Marine Hospital Service. Washington, Government Printing office, 1890. See p. 181.

[21]A Manual of Bacteriology, pp. 886. Illustrated by heliotype and chromo lithographic plates and two hundred and sixty-eight engravings. New York, Wm. Wood & Co., 1892.

[22]Die Pilze.

[23]The Bacteria, an account of their nature and effects, together with systematic description of the species. Eleventh report Board of Trustees, Illinois State University, pp. 92-157.

[24]Genera e. Spec. delle Batteriaceæ, 1889.

[25]Beitraege zur Biologie der Pflanzen, Vol. II, p. 130.

[26]Die Spaltpilze, pp. 101 with 34 figures, Breslau, E. Trewendt. 1884.

[27]Text book of Bacteriology, English translation.

respects they are essentially alike. Prof. Osborn further calls my attention to the fact that in gall insects the character of the gall produced by the insect is of great importance in separating species.

I may be permitted in this connection to briefly quote from several prominent writers on this question. Trelease[28] summarized the characters as follows: 1. Morphological characters, mode of growth in which cultures show full range of variability of each species, behavior of cells to staining fluids, motion of the cells. 2. Physiological characters, production of pigment, specific fermentation and liquefaction of gelatin are apparently reliable. 3. Pathogenic characters for the most part are unreliable to render species which depend at all upon them above suspicion, though they may offer valuable collateral evidence. Any physiological characters therefore to be useful in the delimitation of species of bacteria, must be reasonably constant as well as pronounced. The fact is with our present means of cultivating bacteria, strictly parasitic, like the *Spirochaeta* of relapsing fever; that it grows with great difficulty in artificial cultures, like the Micrococcus of gonorrhoea, that it dies after a short time when cultivated, unless re-innoculated like the swine plague bacillus of the Germans and our Department of Agriculture all the pecularities have at least a suggestive value." Fraenkel[29] writes: "Were the microscopical examination of the bacteria as they occur in their natural state, the only means at our disposal for studying them, our knowledge of bacteriology would never get beyond the experimental stage of certain very narrow limits." H. Marshall Ward[30] in an admirable article says that before new species are described the following points should be clearly made out: 1st. Habitat, air, soil, milk, etc. 2d. Nutrient medium agar, gelatin, potatoes, broth, saccharine liquids, etc. 3d. Gaseous environment, aerobic, anaerobic, whether carbon dioxide, nitrogen or hydrogen affect the growth. 4th. Temperature-optimum is the most important though maximum and minimum should also be recorded. 5th. Morphology and life-history, shape, size, mode of union, presence of sheaths and capsules, spores, endospores and arthrospores, cilia, involution forms, etc. 6th. Special behavior. Does the germ peptonize and liquefy gelatin? 7th. What is the shape and course of the area? What is the shape of the colony? 8th. Pathogenic properties. But before we can do a great deal in this line some general code should be adopted.

From these observations it will be seen that it is not an easy matter to recognize species; partial descriptions must be entirely ignored. I will admit with H. Marshall Ward that some general standard should be set up. But it would seem to me that we should soon begin to do something more on the biological characters of many species. Many of these points in our species are still in a somewhat uncertain state. They have, in fact, not been determined.

Much bacteriological work can be done with little equipment, but the systematic portion of this work can not be done without the literature at hand. To work out our bacteriological flora is needed, but it may be a long time before this work is accomplished. What is needed is a thorough scrutinizing of species to determine how many of these are synonyms. Marshall Ward[31] has attempted this for a good many of the species occurring in water, and he has what appears to me, placed together some species which are distinct. Marshall Ward is however, a most careful investigator, who discriminates with great care. This part of the work can

[28] The Weekly Medical Review, Vol. XIX, March 23, 1889, p. 315. St. Louis

[29] Text Book of Bacteriology.

[30] On the Characters, or Marks, employed for classifiying the Schizomycetes, Annals of Botany, Vol. VI, No. XXI, April, 1892, pp. 103-144.

[31] Philosophical Transactions, 1892 or 1893.

not be done by a novice. I am greatly inclined to believe that many species have been described, as was true in many cases, of early systematic efforts with higher plants, without looking up the literature or carefully comparing specimens. It is out of the question in smaller institutions where library facilities are so meager that they should have access to much of the literature, and this is especially true where many of the species are described in out of the way journals. It seems to me that it would be expedient to describe species only in well recognized journals devoted to this line of work like Zeitschrift fur Hygiene, Centralblatt fur Bakteriologie und Parasitenkunde. The Botanical Gazette, Bulletin of the Torrey Botanical Club, or possibly the American Monthly Microscopical Journal might undertake to do this line of work on this side of the Atlantic.

BACTERIA AND THEIR RELATION TO THE DISEASES IN MAN AND LOWER ANIMALS.

The subject of Bacteriology has become so important in modern medicine that no physician can claim recognition as an authority in zymotic diseases unless he treats it from the standpoint of the modern advancement in this the newest of sciences. The author who ignores the facts of bacteriology can no longer find place as an authority in the library of a physician. Facts are being established however, so rapidly that even the best of works soon become obsolete.

Dr. Baumgarten says[33]: "In a study of diseases, the aetiology must not be considered by itself, when in this case we are dealing with organic beings——, bacteria and animal life, which bear certain relations to each other, the success in treatment cannot be controlled by a single factor."

Patrick Geddes[34], in that most charming of books, Chapters in Modern Botany, says: "Most important, however, is the fact expressed in the germ theory that bacteria are constantly and intimately associated with some of the most fatal of human diseases, such as consumption, diphtheria, small pox, or typhoid, malaria or leprosy. Bacteria, in fact, will kill most of us."

DeBary[35] says: "It is not necessary to enlarge upon the manifold interest attached to these organisms at a time when the statement urged daily on the educated public does not fall short of saying, that a large part of all health and disease in the world is dependent on bacteria."

So long as the old ways of looking at the nature of contagious diseases was in vogue, little could be expected, since it was before the advent of the cotton air filter by Schrœder and Von Dusch (1854) methods of sterilization, used by Schwann and others of his time, and perfected by Pasteur, Koch and modern workers, the use of analine dyes to stain bacteria, the introduction of culture media by Cohn, Pasteur, Brefeld, Schroeter, and the plate method of separating germs first used by Koch; these landmarks have, in a large measure, helped to give us a clear understanding and knowledge of the contagious nature of diseases.

We have seen that several authors believed that diseases like anthrax and cholera were supposed to be carried by specific organisms. In some cases, as in anthrax, Davaine had observed, in 1850, that the blood of anthrax animals contained stiff rods of the anthrax bacillus. Pollender observed the same rods in 1849. In 1863 and 1864 Davaine presented to the French academy the results of his innoculation experi-

[33]Lehrbuch der Pathologischen Mykologie Vorlesungen fur Aerzte und Studirende. pp. 973, with 108 figures, Harald Bruhn. Braunuschweig 1890, see p. VII.

[34]Chapters in Modern Botany, New York, Chas. Scribner's Sons, 1893, pp. 201, with 8 figures.

[35]Lectures on Bacteria, second improved edition, English translation by Henry E. F. Garnsey, revised by Isaac Bayley Balfour, pp. 193 with 20 figures. Clarendon Press, Oxford, 1887.

ments with the blood of diseased animals. It was also shown as early as 1865 that sputum taken from tubercular patients would produce tuberculosis. As yet, however, the evidence was not conclusive. In 1877 Koch published the results of his work on this disease, in which he showed conclusively that this special bacillus, which he had isolated from diseased animals and cultivated outside of the animal body, produced typical anthrax; that in the animal only the vegetative condition occurred, but when the animal dies these rods break up into spores; that infection in cattle and sheep commonly results from the taking up of spores while grazing in an infected pasture. The organism thus lives a dual life, one in the animal and one in the field.

In ordinary cultures, spores are readily formed and these retain their vitality for a long time. The writer has found that these when kept in silk threads retain their vitality for at least six years. We mention this disease in particular because it shows what rules must be followed in bacteriological research. The classic canons of Koch must ever be observed, and these are, first, constant presence of the germ with the disease; second, isolation and cultivation of the germ; third, successful innoculation experiments with the germ isolated, and followed by the same disease; fourth, this germ must be the same as in the original diseased animals. Dr. Russell[36] well says that these canons are just as applicable to phytopathology as in animal diseases. For my own part, I am sorry to say that so many bacterial diseases of plants have been described in which these canons have not been observed. But to follow through in detail the various stages of the history of this part of bacteriology, however interesting it is, would make this paper entirely too long. We shall therefore touch only upon the more important points.

Let us briefly consider the pyogenic organisms and their relation to septic infection. The lengthy disputes between different investigators on the subject of septic infection and the causal relation to the same and definite micro-organisms had a most excellent champion in Weigert,[37] who, in an able paper, set aside the generally accepted theories, that septic infection resulted from poisonous products of ordinary saprophytic germs, or that certain changes occurred in the body before the germs could develop. It was the old story of Justus von Liebig,[38] who strongly argued that germs and fungi follow a diseased condition. Weigert especially emphasized the importance of recognizing bacteria in different diseases. He should receive much credit for having done a great deal towards perfecting methods of staining bacteria.

The pyogenic microbes have been a rich field for investigators. For is not this subject of great importance to the physician? Almost daily he meets with the germs in question. They are concerned in such diseases as septicæmia, pyæmia and erysipelas. Then, too, these cocci are found in diphtheria. The forms of septicæmia occurring in lower animals are numerous, as Koch[39] first showed. A form of *Micrococcus* commonly placed in the genus *Streptococcus* is widely distributed in nature, and also produce septicæmia in lower animals. Dr. V. A. Moore has

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[36]Bacteria in their relation to vegetable tissues. Dissertation presented to the Board of University studies for the degree of Doctor of Philosophy, Johns Hopkins University. Freidenwald Company, Baltimore, 1892; pp. 41.

[37]Ueber pockenaehnliche Gebilde in parenchymatosen Organen und deren Beziehung zu Bacteriencolnien, Breslau, 1875. See Loeffer Die Geschichtliche Entw, etc, p. 203.

[38]Chemistry, in its application to Agriculture and Physiology, edited by Lyon Playfair, Philadelphia. T. B. Peterson, Part Second, pp. 87, 119.

[39]Wundinfectionskrankheiten, Leipzig, 1878. Mith. d. kais- Ges. Amts Vol. I.

isolated twenty-eight species of this genus. Five of them are pathogenic to common mice.[40] Many of these Streptococci are not, however, pyogenic.

Ever since Ogston, Rosenbach and Passet demonstrated the presence of *Staphylococci* and *Streptococci* in pus, it has been universally held that they had some causal relation to the formation of pus. But, it is also a well established fact that pus may be formed without germs as was first demonstrated by Grawitz and later by Scheurlen and others. The aseptic introduction of turpentine, nitrate of silver, and sterilized pus cultures under the skin will give rise to pus. That certain other pathogenic bacilli and some saprophytic bacteria when sterilized can cause the formation of pus seems also to be reasonably well demonstrated. So universally are these pyogenic micro-organisms distributed that unless the greatest precautions are taken, they gain entrance to the wound and, the surgeon finds his patient not recovering as rapidly as he should. These pus organisms have a low thermal death point. The *Streptococcus pyogenes*[41], 52–57.4° C.

Staphylococcus pyogenes var. *aureus*[42] according to Sternberg is killed at 56° C., but Mr. Wade found in the writer's laboratory that it is somewhat higher, perhaps a different race[43]. This is a relatively low thermal death point since many species especially the anthrax bacillus produce resistent spores which stand 100° C. for several minutes. Some of the germs commonly found in the air like *Sarcina lutea* which do not form spores are only destroyed above 70° C. when heated for ten minutes.

There are few diseases which have awakened a deeper interest than tuberculosis in man and lower animals. The announcement of the discovery of the Bacillus was made by Koch[44] in 1882 and independently, about the same time, Baumgarten[45] discovered a specific Bacillus as the cause of tuberculosis. Villemin[46] as early as 1865 had shown that tuberculosis might be induced in healthy animals by innoculation of tuberculous material. These results were later confirmed by Cohnheim,[47] Salomonsen[48] and others. Baumgarten and Koch demonstrated the identity of tuberculosis in bovine animals and man. Later it was shown by Ernst and others[48] that milk from tuberculosis animals was infectious.

There was much hesitancy at first to accept the conclusions of Koch in regard to the infectious nature of tuberculosis, for the theory that tuberculosis was an

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40 Veranus A. Moore in a paper on Miscellaneous Investigations concerning Infectious and Parasitic Diseases of Domesticated animals. Bulletin No. 3, Bureau of Animal Industry, U. S. Dept. of Agriculture, pp. 9-30, gives an interesting account of the biology of some of these Streptecocci and also refers to the work of Smith, Salmon, Rosenbach and others.

41 Sternberg's Manual of Bacteriology, p, 274.

42 l. c. p. 267.

43 It is possible that in this species as in *Bacillus pyocyaneus* there are different races as has been shown by several investigators.

44 Die Aetiologie der Tuberculose, Berlin Klinische Wochenschrift, 1882, No. 5.

45 See Baumgarten Lehrbuch der Pathologischen Mykologie Vorlesungen fur Aerzte und Studirende, Harold Bruhn, Braunschweig, pp. 973 with 100 figures. See page 535.

46 Etude sur la tuberculose, Paris, 1868.

47 Uebertragberkeit der Tuberculose, Berlin, 1877.

48 How far may a cow be tuberculous before her milk becomes dangerous as an article of food, Hatch. Experiment Station Mass. Agricultural College Bulletin No. 8, April, 1890. Bang. Proc. Inter-nat. Medical Congress, Copenhagen, Vol. I., Path. Sect. p. II. 1884. McFadeyean and Woodhead, see Woodhead, Bacteria and their Products, p. 224.

Smith & Schroeder, Bull. No. 3, Bureau of Animal Industry, U. S. Dept. of Agriculture.

A contribution to the question of the danger of infection with tuberculosis through ordinary milk. The Journal of Comp. Path. and Therap., Vol. VI, p. 97.

inherited disease, was too strongly intrenched in the minds of physicians and people generally. But Koch brought such conclusive evidence in his first paper that the contagious nature of the disease could not be doubted and is now almost universally accepted. Physicians to-day use the methods proposed by Koch, Ziehl, Ehrlich and others for determining the presence of tubercle bacilli in sputum, lupus and other forms of the disease. A subject that was widely commented upon a few years ago in the press of the whole civilized world was the discovery of a toxic product, *tuberculin* in cultures of tubercle bacillus. This product discovered by Koch is soluble in glycerine. It is a powerful therapeutical agent. In very minute doses, when injected subcutaneously into tuberculous animals, it produces febrile and other decided symptoms. Dr. Sternberg[50] says: "This discovery must rank as one of the first importance in scientific medicine whatever the final verdict may be as to its therapeutic value in tubercular diseases in man." Numerous experiments have been made to determine its value as an agent in diagnosis of tuberculosis in bovine animals. These investigations have not only been carried on in Europe, but in our own country Dr. Pearson[54] has shown how valuable it is in cases of this kind. I may also refer to the value of another product, *mallein*, which Dr. Theobold Smith[55] and others have used with great success in diagnosis of glanders.

These and other results which have been obtained along the lines of bacteriology have been of inestimable value to the world at large. We cannot overlook the great work of Pasteur in affording immunity to persons bitten by mad dogs. Hydrophobia, that strange malady which has baffled medical skill will, it is to be hoped, be held in check by the work of this savant.

Although the cause of this strange and fatal disease is still a mystery, the benefits resulting from a series of innoculations are beyond dispute.

SUSCEPTIBILITY AND IMMUNITY.

We can now discuss briefly susceptibility and immunity. No question in general medicine and biology is more interesting than those which relate to susceptibility and immunity from disease in plants and animals. Certain animals and plants are much more subject to some diseases than others. Tuberculosis is common to man, bovine animals, apes and small herbivorous animals. Anthrax is most common in cattle and sheep; it may be communicated to man, guinea pigs, rabbits and mice. Rats, dogs, and birds are generally exempt. Glanders is most common in equine animals, occasionally forms a loathsome disease in man, but mice, rabbits and cattle are generally exempt. But this difference of a disease is not confined to different species; it often occurs in different individuals of the same species. Thus hog cholera of the U. S. Department of Agriculture[56] nearly always takes away a majority of the animals, but a few will not take the disease.

A case has come under my observation in which various pathogenic germs were innoculated into a rabbit, but all without avail. Again common laboratory experience shows that very young animals are much more liable to resist diseases than

Weitere Mittheilungen ueber das Tuberkulin, Deutsche Med. Wochenschrift, 1891, No. 43.

50 Manual of Bacteriology, p. 387.

54 Bull. No. 21. Pennsylvania Agrl. Experiment Station. E. P. Niles, Tuberculosis and the Koch test, Virginia Agrl. Exp. Station, vol. II, N. S. No. 3.

55 W. B. Niles, Bull. No. 20, p. 729, Iowa Agrl. Exp. Station.

56 Hog Cholera: Its history, nature and treatment, as determined by the inquiries and investigations of the Bureau of Animal Industry, pp. 199, with 16 plates. Government Printing office, Washington, D. C. See p. 34.

older animals. The same thing holds true in the human race, and very properly the term "children's diseases" is used for a number which are common to children and not older people. In older people some diseases are rapidly fatal, while other persons are exempt. The negro race is much more subject to tuberculous troubles than the white race. Small-pox is much more severe in dark races than fair skinned. The negro and latin races of tropical climates are more exempt from yellow fever than northern people. It is said on good authority that where cholera is indigenous, that the percentage of death is smaller than where it is not.[57]

Dr. Sternberg says:[58] "The tendency of continuous or repeated exposures to the same pathogenic agent will evidently be to establish a race tolerance; and there is reason to believe that such has been the effect in the case of some of the more common infectious diseases of man, which have been noticed to prevail with special severity when first introduced among a virgin population, as in the islands of the Pacific, etc."

In bacterial diseases of plants the same thing has been noticed; every horticulturist is familiar with the fact that some varieties of apples are more subject to the attacks of blight (*Bacillus amylovorus*) than others. It is certain that this susceptibility must depend on certain conditions in the animal body or plant, either favorable or unfavorable for the development of the pathogenic organism. It may be that the temperature fluids, of the body, or the blood serum as Buchner[59], Hankin[60] and others claim have valuable germicidal properties. The products of certain glands like the thymal are said to afford immunity. Fokker[60a] has recently published results which show that fresh milk has germicidal properties. It may be that the tissues of plants or structure of parts of cells, or the fluids of the plant are different from those attacked. Immunity from subsequent attacks varies in different diseases, and the time also varies. The theories advanced for immunity are the exhaustive theory, which holds that the organism growing in the animal exhausts the supply of some substance essential for its growth. But this has been set aside by Sternberg[61] and others.

The retention theory, proposed by Chauveau: This investigator holds that certain products formed during the development of the germ in the body accumulate and are retained. The vital resistance theory of Sternberg[62] explains immunity upon an acquired tolerance to the toxic products of pathogenic bacteria. There is much evidence to support this theory. The theory of phagocytosis, first prominently advocated by Metchnikoff, and sometimes called the Metchnikoff theory, is based on the fact that bacteria in the blood are picked up by the leucocytes. That immunity depends upon the power possessed by these leucocytes in destroying bacteria. There is no longer any doubt that the leucocytes pick up and destroy microorganisms in animals, for since the germs found in these leucocytes are often corroded, and finally disappear entirely when health has been restored. Hankin[63] believes there is found in the body, as a result of disease, antitoxine, and these substances which are found in immune animals, he calls "defensive proteids;" these are clas-

[57] Sims Woodhead, Bacteria and their Products, Chapters VIII and IX.

[58] Manual of Bactierology, p. 227.

[59] Centralblatt fur Bakt. und Parasitenkunde Vol. V, p. 817; Vol. VI, p. 1.

[60] Proc. Royal Soc., London, 1890, May 22.

[60a] Fortschritt der Medicin Vol. VIII, p. 7.

[61] Journal of Medical Sciences, April, 1881; Manual of Bacteriology, p. 238.

[62] American Journal of Medical Sciences, April, 1881. Manual of Bacteriology, p. 240.

[63] See Sternberg's Manual of Bacteriology, p. 260.

sified according to whether they occur in normal animals, *sozins;* second, those occurring in animals which have acquired an immunity, *phylaxins.* Sternberg,[64] than whom there is no higher authority in this country, says: "The experimental evidence detailed gives strong support te the view that acquired immunity depends upon the formation of antitoxine in the bodies of immune animals; as secondary factors, it is probable that tolerance to toxic products of pathogenic bacteria and phagocytosis have considerable importance, but it is evident that the principle role cannot be assigned to these agencies."

Sims Woodhead[65] thus summarizes immunity: "It appears probable that both the antagonistic action and this summative action are due to the bringing into play, or the depressing, of certain specific functions of the protoplasms of the cells by the products of different micro-organisms. It is not necessary that these functions should always be manifesting themselves; after being once evoked and exercised they may remain latent for a considerable period, and only be again called into action under the regular specific stimulus. It is a case of writing on the looking-glass with ink and with French chalk—the ink is always in evidence, and we might say that it corresponds to the enzyme, or the peptonizing functions exerted by certain cells, animal and vegetable, whilst the French chalk, though always there, is only brought out when the glass is breathed upon."

BACTERIA OF THE INTESTINAL TRACT.

In a previous paragraph I referred to studies made by Hallier and others on Asiatic cholera, and the pleomorphism of bacteria. This disease, which for centuries has carried away thousands of human lives every year, is certainly worthy of the deepest and most profound studies of physicians and bacteriologists. That the disease is contagious in its nature has long been recognized. The distinguished investigator, von Pettenkofer, long worked in vain for the specific cause. His work on the spread and distribution of the disease is a most important contribution to the literature of the subject, especially his researches on the relation of ground water and the "drying zone" to cholera epidemics. The splendid achievements of Robert Koch who was sent by the German government in 1883 to study cholera in Egypt and India made his name famous. On this mission he demonstrated a specific micro-organism which he called the "comma bacillus," but which belongs to the spiral forms and is known as *Spirillum cholera asiaticæ.* This germ was found in the dejecta of patients suffering from this disease, in cesspools and water which received the dejecta, in milk, etc. It was not as easy to convince scientists and physicians that the germ found by Koch was the cause of Asiatic cholera, since Finkler and Pryor[66] found a germ in Cholera nostras which appeared to be identical, and Deneke[67] found apparently the same germ in old cheese.

Miller[68] found a comma bacillus in the human mouth; moreover, Klein, an eminent English authority, claimed that Koch's material was entirely harmless. Although the evidence of a specific germ is not so conclusive in this disease as in anthrax and tuberculosis, yet the accidental innoculation of a young physician in

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64 Manual of Bacteriology, p. 262.

65 Bacteria and Their Products, p. 379.

66 Untersuchungen uber cholera nostras. Deut. med. Wochenschr, 1884, No. 36, etc.

67 Ueber eine neue den Choleraspirillen ahnliche Spaltpilzart. Deut. med. Wochenschr, No. 3, 1885.

68 Kommaformiger Bacillus aus der Mundhole. Deut. Med. Woch, 1855, No. 5. Micro-organisms of the Human Mouth. Philadelphia, 1890.

Koch's laboratory in Berlin with this germ, who became sick and had the symptoms of genuine cholera, the experiments of Ferran, Koch, Gamaleia and others with guinea pigs, leave no doubt as to the causal connection of organism and Asiatic cholera. It is generally recognized now as the cause of this disease. There are many apparent anomalies as shown in the distribution of cholera and von Pettenkofer's "ground water theory," which are fully set forth in Dr. Shakespeare's[69] splendid monograph on cholera. If the contagious nature of the disease and the biological questions are taken into account, these conditions can be accounted for. The history and spread of this disease all show how important it is to take heed of sanitary conditions. It shows that the disease spreads most rapidly where effluvia and excreta contaminate the water; food, too, may be an important item. That old habit of using sewage water to sprinkle over vegetables, or the use of night soil for growing vegetables is an extremely dangerous thing.

WATER ANALYSIS.

This brings up the question of making bacteriological analysis of water and in this connection we may discuss typhoid fever. It is a well recognized fact that this disease is caused largely through the use of water and food that contains the active virus. The causal connection of the Koch Eberth bacillus and typhoid fever is generally conceded, but the proofs are not as certain as in some of the other contagious diseases, since bacteriologists have not been successful in producing typical typhoid fever in lower animals. This is not surprising since there are no animals that take this disease as man does. But it is pathogenic to mice and lower animals. A study of the typhoid fever bacillus is not an easy matter since there are several closely related species like *Bacillus coli-communis* which normally occur in the colon of man, other forms of this species occur in dysentery, cholera infantum, catarrhal enteritis, gastro-enteric catarrh, peritonitis and other diseases. Other germs of this general character are quite common in decaying substances, and some are pathogenic. The hog cholera germ, swine plague; the *Bacillus coli-communis* are well known for their pathogenic properties. Dr. Theobold Smith[69] has, however called attention to some important characters of the germs when grown in the fermentation tube, which enables us to separate *coli-communius* from nearly allied forms.

It has long been customary to regard a chemical analysis of water sufficient to determine whether water is good for drinking purposes or not. There seems however, to be a rapidly growing tendency to move along biological lines. I would not underrate chemical analysis, it should go hand in hand with this biological work. There are so many problems that the biologist cannot explain unless the chemist is at his elbow. Dr. Stevens says: "It is perhaps enough to say that a chemist is not of necessity a sanitarian, nor is his work the most important basis upon which a sound or safe conclusion is built as to the proper hygienic value of water for potable uses." Mr. Rafter[74] a well known sanitary engineer says: "Attention should be called moreover to the general proposition that the chemical methods are so refined in their nature that a slight error is liable to invalidate the results; whereas the microscopic analysis has the advantage of making the bulk of the organic contaminating material visible to the sense of sight." The chemist can determine that

[69]Report on cholera in Europe and India, pp. 945, with numerous charts and diagrams. Washington, Government Printing Office, 1890.

[69]Centralbatt fur Bakteriologie und Parasitenkunde, Vol. XII, p. 367.

[74]On the micro-organisms in Hemlock water. The quotation from Stevens is taken from this paper.

there is an organic impurity, the bacteriologist can tell what the impurity is. Bacteriologists have made many analyses of water and sewage. The methods used are still open for improvement. Water analysis is indeed a difficult problem.

Prof. Sedgwick,[75] in an exhaustive treatise on purification of water and sewage in report of the Massachusetts State Board of Health, says: "Although microscopical analyses (so-called) of water or sewage have often enough been undertaken the methods employed have hitherto been so imperfect that little importance has been attached either to the examinations themselves or to the results."

There are two ways in which water may be examined: First, microscopically; second, cultures. The former was the method chiefly in vogue before the use of the Koch system of cultivating germs. This method was employed by Cohn [76] and Radelkofer [77] in making examination in Breslau and Muenich. The bacterial examination of water requires cultures, and this is a very important part of the work. But I do not believe that culture examination is sufficient for this work. The Massachusetts State Board of Health employed Dr. Sedgwick, a well known authority in biological research, to make a biological study of sewage and drinking water. A new method was introduced as the combined work of Kean, Sedgwick and Rafter [78] which makes it a comparatively easy matter to determine approximately the microscopical organisms.

Jœrgensen[70] has well stated that the exclusive use of gelatine in this branch of biology may introduce sources of error. Hansen's work, as well as that of Jœrgensen, was more especially intended for zymotechnical purposes, and yet I believe it is equally applicable in hygiene. It may be well to start a series of cultures in small flasks that contain sterilized sewage or water, with some organic matter. For a study of these germs the Hansen method may be used. I believe that good results may be obtained by using liquid media. Miquel's[71] work certainly shows good results. The use of the fermentation tube, as suggested by Dr. Theobald Smith,[72] is a most excellent device. Many of the bacteria found in faeces are gas generators and by use of the fermentation tube which contains bouillion and sugar, the kind and quantity of gas produced may be determined readily. Stoller[73] has recently used this apparatus extensively with some success in arriving at the quantity of faecal bacteria in water.

The most important methods in bacteriological examination of water are those of the Koch school. In this method a known quantity of water, a fraction of a cubic centimeter is put in gelatin or agar and the number of germs which develop are counted. Obviously the smaller the fraction the more danger there will be of making errors in giving the result of the number of germs per cubic centimeter.

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75 A report of the Biological work of the Lawrence experiment station of Massachusetts State Board of Health, 1888-1890.

76 Ueber den Brunnenfaden (*Chrenothrix polyspora*) mit Bemerkungen ueber die Mikroscopische Analyse des Brunnenwassers, Beitraege zur Biologie der Pflanzen I, p. 108 Breslau 1870.

77 Mikroskopische Untersuchung der Organischen substanzen im Brunnenwasser, Zeitschrift fur Biologie I (1865), p. 26.

78 Experimental investigations, Mass. State Board of Health, 1888, 1890, Pt. II, pp. 803, 811. Recent Progress in Biological Water Analysis, Journal of the New England Water Works Association, September 1889. The Biological Examination of Potable Water, Proceedings Rochester Academy of Sciences, 1890.

70 L c, p 48.

71 Annuaire del' Observatoire de Montsouris 1877-1890. Not seen in the original.

72 Centrallblatt bur Bakteriologie und Parasitenkunde. Vol. VII, p. 302, and Vol XII, p. 367.

73 Science, Vol. XXII, No. 564, p. 286.

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Various bacteriological analyses made in Europe and the United States show that the bacterial contents differ greatly. Dr. Gruber[79] sets the maximum number of colonies to be found in spring water from 40 to 50, in well water 300 to 500 per c. c. Frænkel states that good drinking water should not have more than fifty germs per cubic centimeter. Many bacteriologists place the limit at 1,000 germs per c. c. It is stated that water taken from the Croton reservoir, New York, contained from 5,000 to 15,000 germs per c. c., and Messrs. McCall and Patton found in well water from a well near the Iowa Agricultural College, 320 germs per c. c. Spring college water supply only contained 56 germs per c. c. Water taken from the Muenich supply contained from 305 to 12,606 germs per c. c. Frænkel[80] estimated the number of germs in the water supply of Berlin at 6,140, while below the city there was a great increase, the number being 243,000 per c. c. The Kiel water supply, according to Breuning[80a], has from 62 to 1,712 germs per .5 c. c., the number of liquefying species varying from 4 to 188. Wells in the same city in some cases had more than 26,000.

Sewage, of course, contains an enormous number. Out of 126 analyses of Lawrence sewage, the number was 708,000 per c. c.; the minimum was 102,400; and maximum, 3,963,000. Fourteen analyses show more than 1,000,000 per c. c. It is not strange that sewage should contain such large numbers, since the putrefying material is especially favorable for their development. Nor is it strange that well water should often contain large numbers, since the upper strata of the soil teem with bacteria, and it is especially easy for water from the surface to find its way into the well. In bacteriological analysis of water it is not so important to determine the number as it is the quality of the germ. It is of special importance to take into account the pathogenic organisms, like the typhoid fever bacillus, and the spirillum of Asiatic cholera, in cases of epidemics of the latter disease. The liquefying species, such as peptonize gelatin, are more important than those which do not, since many of these give rise to very disagreeable odors, and perhaps poisonous products. What becomes of the germs found in sewage? It is certainly important to know whether they will continue to contaminate cities using the same water and lying farther down the stream.

Water may be purified in two ways: 1. Self-purification; 2. Purification by filtration. In this paper we are only concerned in the first. Destruction by various small animals, chemical action, sedimentation, and direct sunlight. The chemical action is perhaps due largely to oxidation; the mechanical effects of the small particles in the water must act to a considerable degree on the germs; the sediment carries with it much organic matter; this sediment, as experiments have shown, contain pathogenic germs. Perhaps the most powerful agent is sunlight. Buchner,[81] Marshall Ward, and others, have shown that exposure of typhoid bacillus, anthrax and other germs to direct sunlight destroys their pathogenic properties and inhibits their growth very materially. That there is a constant decrease in the number of germs at some distance below the point where sewage empties into the stream, numerous analyses have shown.

79Schrank. Anleitung zur Ausfuehrung bacteriologischer Untersuchungen zum Gebrauche fur Aerzte, Thieraerzte, Nahturungsmittel—, Agricultur und Gaehrungschemiker, Apotheker und Bautechniker, pp. 255, with 137 figures. Leipzig und Vienna. Franz Deuhicke, 1894.

80l. c. p. 820.

80aBacteriologische Untersuchung des Trinkwassers derStadt Kiel im August und September, 1887. Inaugural Diss., pp. 38. Kiel, A. F. Jensen.

81 Bot. Centralblatt Vol. LII., pp. 61, 398.

DISEASES OF PLANTS AND INSECTS.

In this lengthy sketch on pathogenic germs the relations to hygiene have been touched on sufficiently. I have not discussed many of the diseases, but with such a vast subject, it is impossible to do so. Before I proceed to discuss the uses of bacteria to agriculture, let me briefly refer to a few of the diseases they cause in plants. Scarcely a decade ago DeBary,[82] Hartig[83] and other phytopathologists believed that the acid reaction of higher plants was detrimental to the growth of bacteria in living tissues. Since then it has been shown that many bacteria find acid media an excellent medium; moreover European, but more especially American investigations have shown that quite a number of plant diseases are caused by these minute organisms. The pioneer work in fact in this direction was paved by Americans. Most European authors like Kramer[84] and other bacteriologists scarcely enumerate the work done by Americans.

The only writers who have fully comprehended the subject are Ludwig of Greiz,[85] and Comes[86], of Italy, yet more than a decade ago Professor Burrill[87] worked out the causal relation between pear blight and *Bacillus amylovorus*. This was soon followed by the work of Prof. Arthur[88] on the same disease, and finally some excellent work by Waite. Then followed the investigation of Burrill[89] on sorghum blight, the work of Kellerman and Swingle[90] on the same disease. Tuberculosis of the olive by Savastano[91], blight in oats by Prof. Galloway[92] and Wakker's[93] Yellows of Hyacinths has become quite familiar to phytopathologists of Europe.

It has been demonstrated that there are other plant diseases caused by micro-organisms. These have been tabulated in an interesting paper by Dr. Russell[94].

Not the least value may be expected from the part that micro-organisms play in causing diseases of insects. Flacherie of the silkworm (*Streptococcus bombycis*) long ago studied by Bechamp[95] and Pebrine (*Nosema bombycis*) discovered by Cornalia and carefully studied by Pasteur and Naegeli are the oldest among the known diseases caused by bacteria. Both are most troublesome enemies of silk culture. Pasteur rendered this industry most important aids in suggesting the separation of the moths in pairs in isolated numbered cells, and a microscopical examination of the mates after they had deposited their eggs. The eggs from diseased insects are not to be used for breeding purposes. Whether this organism is to be classed with Bacteria or is one of the *Sporozoa* is still undetermined. Metchnikoff classifies it with *Sporozoa*.

Foul brood of bees, a most troublesome disease in the apiary, is caused by *Bacillus*

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[82] Lectures on Bacteria, 1887.

[83] Lehrbuch der Baumkrankheiten.

[84] Die Bakteriologie in ihren Beziehungen zur Landwirtschaft und den Landw, Technischen Gewerben. Pt. I, pp. 171. Pt. II, pp. 178. Carl Gerold's Sohn Vienna. 1890-1892.

[85] Lehrbuch der niederen Kryptogamen, 1892.

[88] Annual Report New York State Agr. Experiment Station, 1884, p. 357.

[89] Eighth Ann. Meeting Soc. Prom. Agrl. Sci., p. 30.

[90] Annual Report Kansas Agr. Experiment Station, 1889.

[91] Ann. D. R. Scuola. Sup. d'Agri. in Portici, Vol. V, fasc. IV, 1887.

[92] Journal of Mycology, vol. VI, 1890.

[93] Bot. Centralblatt, Vol. XIV, 1883, p. 315.

[94] l. c. pp. 35-41.

[95] Bechamp.: Compt rend., Vol. LXIV.

Pasteur: Etudes sur les Maladies des vers a soie, Paris, 1870.

Balbiani, Lecons, sur les Sporozoairies, Paris, 1884.

alvei. The causal connection of this germ and "foul blood" was first established by Watson Cheyne.[96]

Many bacterial diseases of insects are beneficial, like "flacherie" of the cabbage butterfly (*Pieris rapae*) the bacterial disease of "chinch bugs" (*Streptococus insectoruu*) carries large numbers of this troublesome pest away. In this country Prof. Forbes[97] was the first to study "flacherie" and other bacterial diseases of insects. That these spread rapidly was shown by Prof. Osborn[98] who introduced diseased worms of the cabbage butterfly from Illinois. Later, C. V. Riley.[99] and under him F. W. Mally,[100] carried on some experiments with contagious germs to determine whether the "boll worm" could be held in check. Prof. Snow[101] of the University of Kansas, has also carried on a long series of experiments with the "chinch bug" disease. From the results obtained by these investigators there is no doubt that if the germs are carried over successfully either by the insects, or cultivated in nutrient media, that they may be utilized with advantage. Of course the insects must be gregarious, so that the disease can be spread easily. It is too soon to make any general predictions concerning the application of this work in holding insects in check, but we may confidently expect that it will find application in applied entomology.

We may note in this connection that Loeffler has successfully spread a disease of field mice, *Bacillus typhi-murinum*, in Southern Russia, and in this way materially checked this plague.

BACTERIA OF SOIL.

Let us briefly turn our attention now to a consideration of the bacteria of soil and the decomposition of organic matter, the formation of nitrates and nitrites. It has well been said that while bacteria cause much misery in the world they are great benefactors. Without them there would hardly be any rot nor decay. Our beautiful landscapes could not exist. The earth, garnished with the bloom of flowers, the green herb, its magnificent forests, our cereals and food plants, would not have the material from which to build up their fabric, except for these tiny plants. The nitrogen so essential for all living plants is only made ready for the use of most green plants by these wonderful micro-organisms.

Nitrification formerly meant the production of niter, a natural product of certain soils and rocks, but modern chemists have given to the word a wider meaning. It concerns the formation of nitrates and nitrites.

The older theories are discussed in various works on agricultural chemistry[102].

The first suggestion that nitrification was caused by a ferment was made by Mueller[103], but the true nature of nitrification was worked out by the French

96 Frank R. Chesire and Watson Cheyne, Journal of the Royal Microscopical Soc. 1885, p. 11.

J. J. MacKenzie, The Foul Brood Bacillus, *B. alvei;* its vitality and development' 18th Annual Report Ontario Agricultural College and Exp. Farm, 1892, pp. 267-273.

97 Contagious diseases of insects, Ill. State Laboratory of Natural History. Bulletin

98 Iowa Horticultural Report, 1885, Insect Life, Vol. III, p. 143.

99 The Outlook for Applied Entomology, Insect Life, Vol. III., p. 197.

100 Report on Boll Worm of Cotton, Bull. No. 29, Division of Entomology U. S. Department of Agriculture, 1893.

101 Insect Life, Vol. III., p. 279.

102 Johnson, How crops grow, p. 391, New York, Orange Judd Co., 1888; Storer, Agriculture in its relation to chemistry, etc., etc.; Warrington, six lectures on the investigations at Rothansted Experimental Station, delivered under the provisions of the Lawes Agricultural Trust, before the Ass. Am. Agrl. College and Experiment Stations, Washington, Aug. 12-18, 1891; Experiment Station Bulletin No. 8, office of Experiment Stations U. S. Dept. of Agrl., Washington, Government Printing office.

103 Landw. Versuchs Stat. Vol. XVI, p. 233.

chemists, Schloesing and Muentz[104], who announced, in 1877, that they had established, by a series of experiments, that nitrates in the soil were formed by a micro-organism. They showed that 212 degrees Fahr. for one hour was sufficient to destroy the agent that caused nitrification. Further experiments made by these investigators show the importance of taking into consideration the temperature of the soil. In summer the temperature is more favorable for nitrification. The absence of strong light is a necessary condition for this same process. An alkaline condition of the medium is essential, but the amount, as Warrington says, is injurious if anything beyond a small proportion, and a large amount will prevent the action altogether.

The present theory of nitrification is that there are two stages, and each process is brought about by a distinct organism. At least this is true in the nitrification of ammonia, and the nitrification of nitrogenous matter falls under the same head. Warrington,[105] in an admirable paper, says: "By one organism the ammonia is converted into nitrites; by the other the nitrite is converted into nitrate. The existence of these two distinct agents, each of which has special conditions favorable or unfavorable to its development, explains at once the particular formation of nitrous or nitric acid, so frequently observed in laboratory experiments on nitrification." In the soil these two different organisms are abundant; the conditions for their growth being similar, they work together. The most interesting point in connection with these organisms is their growth in nutrient media. Isolation has been attended with much difficulty. The first attempt to grow them was made by Schloesing and Muentz; although they may have had the nitrifying agent, they worked with material that contained other germs. Koch's methods of growing bacteria in solid media, like agar and gelatin, wholly failed to accomplish the desired result. The first success in cultivating the nitrifying organism was made by D. P. F. Frankland.[106] His cultures were started in an ammonical solution, and by the dilution method he finally succeeded in obtaining a single species.

Warrington[107] by the same method succeeded in isolating the organism in the same way. Winogradsky[108] also succeeded in isolating and growing the germ. So much for the isolation of the nitrous organism. The separation of the nitric organism has been attended with equal difficulty, but Winogradsky[109] by an ingenious method has succeeded in growing the nitric organism on gelatinous silica. A most interesting feature of these organisms, the nitrous and nitric, is that they grow in inorganic fluids. Warrington[110] says: "That an organism unprovided with chlorophyll and growing in darkness, should be able to construct organic matter out of ammoniacal carbonate is certainly of the highest interest." Connected with the subject of nitrification is that of denitrification. Numerous investigators have called attention to the breaking up of nitrates in sewage. In some cases as in *Bacterium denitrificans*[111] the nitrate is changed into nitrogen gas. But these nitrogen gas species are evidently not common. The species which reduces the nitrates are numerous as shown by various recent investigations.

104 Compt. Rend. Vol. LXXXIV, p. 301.
105 l. c., p. 63.
106 Phil. Trans. Roy. Soc., 1890. B., p. 107.
107 Transactions Chem. Soc. 1891, p. 502.
108 Ann. d l' Institut Pasteur, 1890, p. 213.
109 Compt. Rend., Vol. CXIII, 1891. p. 89.
110 J. M. N. Munro, Trans. Chem. Soc., 1886, p. 651.
Warrington l. c. p. 49.
Winogradsky, Ann. d l' Institut Pasteur, 1890, p. 268.
111 Gayon and Dupetit, Ann. de la Science Agronomique I (1885), p. 226.

Warrington found 37; Jordan[112] has also found several in the sewage of Boston water supply. Prof. G. E. Patrick made an examination for the writer of eight species; of these five were energetic reducers of nitrates to nitrites. This property was not confined to facultative anaerobes. *Sarcina lutea, Streptococcus cinnabareus* are both aerobic, and yet are energetic reducers.

This field of bacteriology is a most fascinating and an important one. The whole subject of decomposition of organic matter might well engage the attention of many investigators. The results of Schlœsing and Muentz on nitrification and the erosion of rocks through the agents of bacteria, the brilliant achievements of Winogradsky, Warrington and others on these questions should be brought to the attention of agriculturists. These problems are important in the production of crops, and may well stimulate for a knowledge of things that seem hidden.

Let us now consider the appropriation of nitrogen in leguminous plants. Leguminous plants as renovators of our soils has been an established axiom in agriculture for years, but it is only within recent times that this was properly accounted for. Did not Boussingault show that plants cannot take up the free nitrogen of the air through the leaves of plants?

Scientists generally opposed Ville's idea that some plants have the power of taking up free nitrogen, but after nearly half a century of investigation, the world at large has come to accept his conclusions. The various phases of the appropriation of atmospheric nitrogen because of the nitrogen found in the tubercles, and the symbiotic relation to the plants in question, has received wide discussion in the agricultural and scientific papers. It is because the economic and scientific phases are so important and interesting from practical and chemico-physiological standpoints that they have been considered in this way. The practical farmer is interested in the accumulation of nitrogen in soil through the decay of tubercles and the appropriation of nitrogen by the plant. It makes his soil more productive. The chemist and biologist are interested in finding out facts in regard to how this is accomplished, the structure, form and relationship of the organisms in question.

I presume most of you are familiar with the earlier work. At one time they were supposed to be insect galls. Bivona[113] thought they were fungi and placed them in the genus *Selerotium.* Tulasne, with his great knowledge of fungi, cast them out of this group of plants. Later they were held to be normal structures of the plants, "swollen lateral roots," "imperfect buds," normal structures of the roots for the storage of reserve food material. Prof. Atkinson,[114] who has made a most excellent summary of the investigations, reviews the status of the question in three periods, early, middle and recent. During the middle period the preponderance of evidence seems to have been to regard them as normal structures for the storage of reserve food material, although the views of some authors were diametrically opposed. Frank, who at first supposed them to be fungi, related to the genus *Protomyces*, established by De Bary, later entirely abandoned this view and thought they were simply for the storage of proteid material. In this he was supported by Brunchorst, Tschirch and Van Tieghem. Woronin, Kny and others held that they were living structures related to *Plasmodium brassicæ.* Later

[112] l. c.

[113] Quoted by Atkinson. Contribution to the Biology of the organism causing leguminous tubercles, Bot. Gazette. Vol. XVIII, pp. 158, 226, 257, where there is a most excellent bibliography. There is also a good review by Conn. Experiment Station Record, Vol. II, pp. 686-693.

[114] l. c.

researches made by Ward[115], Hellriegel and Wilfarth[116], Laws and Gilbert[117], Beyerinck[118], Prazmowski[119], Laurent[120], Frank[121], Atkinson, and a host of others, leaves no doubt as to the organisms found in the tubercles.

The results of these later investigations show that in sterilized soil, leguminous plants make but little growth and the tubercles will not develop. The results have been further supplemented by the successful culture of the organisms by Frank, Prazmowski, Laurent, Atkinson and others. There is much conflicting testimony as to the true nature of the changes produced and the structure of the organism. Atkinson says: "The important question is, can these various conflicting notions of the biology of the microsymbiont be harmonized? Leaving out of consideration for the present the real nature of the organism it will be admitted by those who take the trouble to familiarize themselves with the scope of the work covered by the most important investigations that the organism in question consists of an elongated thread-like structure, which branches freely within the tubercle and possesses enlarged portions which present a more or less finely lobed surface; and very much smaller forms which must exist to some extent within the tubercle, are capable of multiplying in artificial media, and when transplanted from artificial media to the roots of leguminous plants, are capable under these more natural conditions and the stimulus of the microsymbiont, of growing out again into the threadlike structures."

As to the place of the organism in the system of plants there is much diversity of opinion. Laurent, as well as Ward, concluded that they were not bacteria but low fungi. Atkinson says: "While in some characters, as noted above, the tubercle organism is very much like *Claodochytrium tenue*, yet in the sum of essential characters it departs too widely from that genus, so that even if it should eventually be clearly shown to be one of the *Chytridiaceæ*, it would still be referable to *Phytomyxa*."

Frank, Prazmowski, and others placed it with bacteria.

Whatever the final disposition will be, Atkinson, it seems to me, has good grounds for calling it *Phytomyxa*.

It is not my purpose to discuss at length the chemical problem, but it may be well to give the opinions of the more recent investigations. J. H. Gilbert[122] says: "The facts at command did not favor the idea that the plant was enabled to fix this free nitrogen by its leaves. It seemed more consistent, both with experimental results and with general ideas, to suppose that the nodule bacteria fixed free nitrogen within the plant, and that the higher plant absorbed the nitrogenous compounds produced." Atwater and Woods[123], while they show that there is an acqui-

115 On the tubercular swellings on the roots of Vicia Faba. Phil. Trans. Royal Society, CLXXVIII (1887), pp. 139.

116 Untersuchungen ueber die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheft z. d. Zeitschr. f. d. Rubenzucker Ind. d. D. R. Berlin, Nov., 1888. Review in Bot., Central b. XXXIX. (1889). 138.

117 On the present question of the sources of the nitrogen of vegetation, etc. Phil. Trans. Royal Society, CLXXX. B. 1-107.

118 Die Papilionaceenknoellchen, Bot. Zeit. 1888, p. 725-735, 741-750, 757-771, 780-790, 797-804.

119 Das Wesen und die biologische Bedeutung der Wurzelknœllchen der Erbse. Bot. Central b. XXXIX. (1889). 356-362.

120 Ann. d. l'Institut Pasteur. V. (1891). 105-139.

121 Ueber die Pilzsymbiose der Leguminoseen. Berlin, 1890.

122 Experiment Station Record, Vol. III, p. 333.

123 Atmospheric nitrogen as plant food. Bull. No. 5, Storr's School Agrl. Exp. Station, Conn., Oct., 1889.

sition of nitrogen in leguminous plants above that found in the soil, are certain of the symbiotic relation of the plant and organism. They leave the question how it is done an unsolved problem. Nobbe, Schmid, Hiltner and Hotter[124] are of the opinion that the nitrogen which the plant contains comes from metabolic processes.

Whatever the future may decide, it is certain that the tubercles are widely distributed on exotic and indigenous, leguminous plants[125].

The ground seems to be gaining that certain low forms of plants[126], including bacteria, have the power of greatly enriching the soil in nitrogen, and we may add that Frank believes that many higher plants can appropriate free nitrogen without tubercles. Frank's general conclusions are not generally accepted by botanists and agricultural chemists.

We have another most interesting case of symbiosis among bacteria. Professor H. Marshall Ward[127] who studied the fermentation of ginger beer finds that a number of micro-organisms are concerned in this fermentation. Ginger beer as most of you know is made by adding to saccharine solutions a quantity of ginger, and a ferment, the latter changes to an effervescing beverage. This alcoholic and viscous fermentation contain moulds, yeast-fungi and a constant bacterium. The yeast-fungus concerned in this fermentation is *Saccharmgees pyriformis*, the Schizomycete is *Bacterium vermiforme*. This according to Prof. Ward originates from the ginger. The vermiform bacterium is enclosed in hyaline, swollen gelatinous sheaths. This organism imprisons the yeast. The anaerobic bacterium only produces the gelatinous sheaths in saccharine liquid in the absence of oxygen. Now Ward has shown experimentally that only when these two species occur together can the ginger beer be produced.

BACTERIA IN THE DAIRY.

One of the greatest achievements in modern science is the application of scientific principles and utilize them in the arts and industries. Since time immemorial yeast has been used for the manufacture of beer[128], known to the ancients as barley or Pelusian wine. Its manufacture evidently spread from Egypt over Europe. Much advancement has been made. Beginning with Pasteur's Studies on Fermentations, the subject was treated from a rational and scientific standpoint, culminating in the brilliant researches of Emil Christian Hansen and Joergensen of the Copenhagen school. The nomadic tribes of Tartary since time immemorial have prepared a fermented drink from mares' milk known as koumiss. The kefir, another fermented drink of milk has long been made by the inhabitants of the Caucasus. Scientists were made familiar with this drink as early as 1784, but it devolved upon modern scientific investigation to rationally explain the causes of this fermentation. There are other ways in which a study of bacteriology is rendering important aid to our modern industries. We need not go far back in the history of bacteriology when it was supposed that the souring of milk was a purely chemical process. Sheele had discovered lactic acid in whey in 1780. Pelouze and Guy Lussac

[124] Landw. Vers. Stat., Vol. XXIX, pp. 327-354.

[125] H. L. Bolley, Agricultural Science, Vol. VII, p. 58; records them on twenty-eight indigenous and sixteen exotic plants in North Dakota.

[126] Berthelot. Compt. rend., Vol. CXVI, pp. 841-849. Experiment Station Record Vol. IV, p. 854.

[127] The ginger beer plant and the organisms composing it. Phil. Trans. Roy. Soc Vol. CLXXXIII. p. 125.

[128] Pasteur, Studies on Fermentation. The Diseases of Beer, their causes, and the means of preventing them. English translation, Faulkner and Robt. Landon. Macmillan & Co , 1879, p. 418, with 85 figures and 12 plates, see pages 1 and 17.

solated lactic acid in milk in 1833; Turpin in 1837, supposed that the cause of souring milk came from the mammary gland and was contained in the fat globules. Schwann and Latour, 1837, had laid the foundations to rationally explain the process of fermentation, making it certain that organized living beings caused the changes observed in a fermenting substance. Fuchs[129] was the first in modern times to examine milk microscopically. He found two germs; one he termed monas and the other infusor. Blandeau, 1847, incorrectly ascribed lactic acid fermentation to yeast (*Torula*) and the common blue mould (*Penicillium*). Liebig supposed that fermentation was a property of all albuminoids and this view gained credence in many quarters. But we must pass over these stumbling blocks in the history of this work and give in rapid succession the vital points which have made it possible to put the fermentations of milk on a high road to a successful use in practice. Pasteur, in 1837, thought souring of milk was due to an organized *Ferment lactique;* he also recognized that other organisms were present; to distinguish the two, he called it *Leveure lactique* caused by his *Vibrio butyricus*[130]. This germ was capable of standing a much higher temperature than the lactic acid organisms. In 1874 Lister, by using bacteriological methods, separated his *Bacillus lactis*, which we have seen led him to erroneous ideas.

Hueppe[131] somewhat later, 1884, made a thorough study of souring milk and referred Lister's *Bacillus lactis* to one which he described as *Bacillus acidi lactici.* In a second paper he concluded that souring was not caused alone by this species, but several. Marpmann,[132] Conn,[133] Storch,[134] Weigmann[135] and others have all shown that species of lactic acid germs are numerous. The power of changing milk sugar to lactic acid is not confined to Saprophytic species, but some of the pathogenic, like the Micrococcus [of osteo-myelitis[136] has the power coagulating the casein of milk. Some of the chromogenes are very active in this direction. The *Bacillus prodigiosus* which often causes red milk in Europe, has this power. It is the famous blood-portent, connected with several superstitions, and certain lesions of the teats, which were supposed to cause bloody milk, is due to nothing more than the development of this bacterium, which may form lactic acid. Schottelius and Wood[137] have pointed out the interesting fact that as the temperature rises the power of forming pigment is lost "and, if it is grown on potato or bread paste, for example, in an incubator at blood heat instead of at the temperature of the room, the color is gradually lost and the culture no longer smells of herring brine, but the power of forming lactic acid from milk sugar, with the accompanying precipitation of casein, is frequently increased, so that it would appear that the energy required for building up pigment was, in this case, directed

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129 Mag. f. d. Ges. Thierheilkunde. 1841.

130 Hoffmann, 1869, also described two species, a motile and a non-motile; the latter he thought caused the souring of milk.

131 Untersuchungen uber die Zersetzungen der Milch durch Mikro-organismen mitth, aus dem K, Gesundheitsamte, Vol. II., 1884, Deutsche Med, Wochenscrh, 1884, No. 48.

132 Ueber die Erreger der Milchsaeure Gaehrung Ergaenzungshefte, Z., Centrallblatt f Allg, Gesundheitspflege, Vol. II., p. 117.

133 Storrs' School, Conn, Agr. Exp. Station, 1889, p. 82; 1890, p. 136; 1891, p. 192.

134 Nogle Undersogelser over Flodens Syrning, etc.

135 Die Bakteriologie im Dieuste der Milchwirtschaft Milch Zeitung, 1891, Nos. 19 and 20.

136 Krause, see Alfred Jorgensen Micro-organisms and Fermentation, English translation, p. 63.

137 Biologische Untersuchungen ueber den Mikrokkus prodigiosus, Leipzig, 1887, p 185. See Sims Woolhead Bacteria, etc., p. 11.

into another channel, and lactic acid and, perhaps, other substances are produced in place of the usual pigment."

Investigation has shown that the flora of milk is a variable one, owing to circumstances under which they make their entrance.

The normal milk from a healthy cow contains no germs. This is easily determined by using a sterilized catheter. The pails and water used to clean milking vessels and cans, the stable, hair from cows, and hands of milker, all have germs that find their way into the milk. The species found are not only abundant, as shown by various bacteriological studies of milk, but both good and bad occur.

Cnopf and Escherich,[137] found from 60,000 to 100,000 per c. c., in milk a few hours after milking. Mr. B. F. White, in the writer's laboratory, found that when milk was obtained in the ordinary way, and cultures made soon thereafter, it contained 40,000 germs per c. c. Milk coming to the creamery had, in some cases, as high as 1,976,000 per c. c. Prof. Conn[138] interestingly shows the enormous number in milk, as well as the great increase. The writer[139] has also brought together the results obtained by Miquel, Weigmann, and others, on the enormous increase, when milk is kept under favorable conditions for their development. That our milk supply of cities contains an enormous number has been shown by Sedgwick and Batchelder.[140] It is not to be wondered at that milk will sour in the course of a few hours on a hot day in summer.

The fact that different samples of milk left standing in a warm room will develop quite different odors is due to particular germs. The practical dairyman is well aware that he cannot always make butter of uniform quality, and this is owing to injurious species. Experiments made during the last few years have shown that by Pasteurizing milk and using the germs that have the right odor, butter of uniform and high quality may be produced. These results were first brought to notice by Storch, of Copenhagen. Weigmann, of Kiel, has also experimented with these germs in a practical way, sending them out to creameries. Prof. Conn, of Middletown, Conn., writes me that he has had success in using one of his own germs.

No one questions the fact that odors and products of bacteria are very characteristic. Storch has called attention to butter that had a flavor of beets, but the animal from which the milk came had never been fed on beets. Dr. Jansen[141] refers to a bacillus which was found in milk that produced a very fetid odor, his *Bacillus fœtidus.*

The writer has isolated a Bacillus which he has called *Bacillus aromaticus*,[142] because of the powerful volatile odor produced. In some media it has an odor characteristic of walnuts. Again it resembles limburger cheese, and a more interesting fact is that it tastes like cheese.

The importance of bacteria in ripening cream is very important, since cheese will not ripen unless Bacteria are present. Duclaux,[143] Adametz,[144] Freudenreich,[145] and

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137 Abst. Centralb. Agrl. Chm., 1890, p. 575.

138 The Fermentations of Milk, Office of Experiment Stations. Bull. No. 9, U. S. Dept. of Agrl pp. 75, see p. 30.

139 The Bacteria of Milk, Cream and Cheese. Report Fifteenth Annual Convention of the Iowa Dairy Association, held at Waverly, 1891, p. 81.

140 A Bacteriological Examination of the Boston Milk Supply. Boston Med. and Surgical Journal, 1892, p. 25.

141 Centralblatt Bakt u Parasitenkunde, Vol. XI p. 409.

142 Bull. No. 21, Iowa Agrl. Experiment Station, pp. 792-796.

143 Le Lait etudes chimiques et microbiologiques, Paris, 1887.

144 Bakteriologische Unteruschungen ueber den Reifungsprocess der Kæse Landw Jahrbucher, Vol. XVIII. p. 228.

145 Landw, Jahrbuch der Schweiz, Vol. V, p. 16, Vol. IV, p. 17.

others have studied the flora of cheese. All find an abundance of bacteria present. They are ærobic and anærobic. Bacteria are very important to the cheesemaker. Cheese without bacteria cannot be made. First of all, in most cases it is necessary for the milk to sour so that the whey can be removed. Again it must pass through a stage of ripening before it becomes digestible. The species differ for different kinds of cheese, and there are several kinds connected with every cheese. As in milk, cheese has its enemies in bacteria. Some that cause abnormal ripening, or color it black, yellow or red.[144] Bacteria always play an important part in the formation of Koumiss. Kefir and other alcoholic fermentations come from Asia and Europe. Mix has shown that forms of alcoholic fermentation of milk occur in North America. The so-called Kephir grains contain the organisms essential for fermented drink Kephir. Yeasts and bacteria have been found. Kern[144] considers that *Diospora caucasica* causes the fermentation. Recent investigations leave much doubt in regard to its being an organism at all. Little is known concerning Koumiss, but that it is caused by some living ferment cannot be doubted. The nomadic tribes of Tartary prepared it from mares' milk, which readily undergoes alcoholic fermentation, Ordinarily it is prepared by adding a little Koumiss or sour, to the sweet milk.

Another interesting group of organisms found in milk are the slime forming bacteria. These organisms cause milk to become very viscous and 'ropy.' It can be drawn out in long threads. This slime, a product from the cell-wall, is analagous to the zoogloea formation in certain bacteria, and comes from the decomposition of sugar. Some of the species that can cause this are *Bacillus mesentericus*, *B. viscosus*, *and Micrococcus discosus*, Bechamp the so-called Frog-spawn (*Leuconostoc mesenterioides*) found in molasses, etc. The species are not uncommon.

Lastly I should mention that bacteria are indispensable to housewives in the making of bread. In this case they are aided very materially by yeasts. Miss Golden[149] has made a contribution to our knowledge of this process and the role bacteria play in bread-making. Miss Golden concludes that bacteria as well as yeast separately can cause bread to rise but that both usually act together. Laurent[148] believes that his *Bacillius panificans* causes the rising of bread besides forming lactic, acetic, and butyric acids.

In conclusion, you will pardon me for having consumed so much of your time. In fact as I look over this question I cannot but think that the subject is so vast that one address will scarcely touch upon the many important problems. The subject of ptomaines and various products of bacteria, disinfection and other points have not been touched up, except incidentally. I venture to say that any one of the topics taken up in this address might very appropriately have consumed the entire time. I shall, however, feel repaid in preparation of this paper if some of the popular notions concerning these baneful and useful organisms, stand corrected.

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[144] Adametz ueber die ursachen und erreger der abnormalen Reifungsvorgange beim Kæse pp. 70. with 6 illustrations. Bremen, 1893. M. Heinsius nachfolger.

[145] Contributions from the Cryptogamic Laboratory, Harvard University.

[146] Ueber ein Milchferment, Bot, Zietung, 1882, p. 264.

[149] Bot. Gazette, Vol. XV, p. 204.

[148] See Centralblatt f. Bakt. und Parasitenkunde, 1887, p. 504.