# PRESIDENTIAL ADDRESS.

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## BACTERIA IN THEIR RELATION TO PLANT PATHOLOGY.

I have chosen the subject of my address with some misgiving and hope to disarm criticism by an instant confession of wrong-doing. I fear that a consideration of Bacteria in their relation to Plant Pathology will hardly be regarded as a legitimate subject of discussion, by the Mycological Society. I may claim, however, this justification: that it is a question much neglected among English Botanists; the ordinary text-books on Bacteria, while dealing fully with the well-known forms pathogenic to animals, make only brief and imperfect mention of those pathogenic to plants, and a brief survey of our present knowledge of this important branch of phytopathology may not be without its use.

When we reflect upon the enormous advances which have been made in recent years in the ætiology of plant-diseases, upon the far-reaching results of pathological researches, which have been pursued hand in hand with modern methods of experiment in associated problems of physiology, it is suprising that little more than 40 years carry us back to the beginnings of this department of science. Somewhere about the year 1860 the older systematists began to be succeeded by mycologists eager to investigate the plant as a living organism. Much splendid work was done at this time by the Tulasnes and other observers in working out the life-histories of various types of Fungi, and invaluable material was left as a heritage upon which to build in the future. In 1865 de Bary published his observations showing the actual entrance of the germ-tube of a parasitic fungus into its host, and his epoch-making researches may truly be said to have laid the foundation of our present knowledge of both plant and animal pathology.

Cohn was one of the earliest workers in the domain of Bacteriology, and he must certainly be regarded as the real founder of this branch of mycology. Koch owed much to his training under Cohn; the latter had already in part worked out the life-history of the *Bacillus anthracis*, and his joy was great when Koch triumphantly demonstrated to him in the Botanical

Laboratory at Breslau the complete pathological development of this parasite. The result of his researches, embodying the first proof that a specific disease of the higher animals owed its origin to a bacillus, was published by Koch in the "Beiträge zur Biologie der Pflanzen" in 1876. In 1878 came Burril's paper on Pear Blight, followed in 1879 by Prillieux's description of the Pink discolouration of Wheat due to a *Micrococcus;* these being the very first accounts of any disease of plants attributed to Bacteria.

Burril traced the disease known as "Pear Blight" or "Fire Blight," producing a blackening of the parts affected and a gummy exudation, to the attack of a micro-organism, *Micrococcus amylovorus.* There was no trace whatever of fungoid growth in the diseased tissues until after the death of the cells. He succeeded in communicating the disease by a series of inoculations by direct infection from the diseased to the healthy tissues, and these results were subsequently confirmed by the more definite cultural experiments of Arthur (1885). It was not, however, until ten years later that Waite fully substantiated the origin of the disease by isolation of the Bacterium and successful infection with pure cultures.

Prillieux made a very close observation of the microscopic features of the disease of the Wheat, which was invariably associated with the presence of *Micrococcus tritici.* He noted their destructive action upon the elements constituting the grain, the corrosion first of the starch-grains, then the proteids, and also the dissolution of the cellulose, but he made no cultures or attempts at inoculation. In the same year (1879) van Tieghem carried out some important investigations for that day upon the action of bacteria as agents in the destruction of cellulose, and claimed that his experiments proved the destruction of the cell-walls in living plants and tubers to be due specially to the influence of *B. Amylobacter.* It is now recognized that he was working with impure cultures. His main contention, however, still holds good, and more recent researches have fully demonstrated the existence of definite bacteria which exercise a fermentative action upon the cell-wall. Wakker's extended investigations (1883-1889) into the nature of the "Yellow-stripe" of Hyacinths led the way in the study of an interesting type of bacterial parasitism, involving a destruction of the tissues which advances along the vascular bundles. A feature of this disease is a blocking of the xylem vessels by a yellow, gummy substance, followed by the dissolution of the cellulose walls. Frequent inoculations always produced a recurrence of the same symptomatic characters in healthy plants; but these were the result of direct infection experiments without the intervention of culture media.

Unfortunately much of this early work rested upon evidence which could not be regarded as conclusive, owing to imperfect methods of experiment and the absence of proper precautions to ensure pure cultures. A knowledge of good cultural conditions, however, was not wanting even at this date, though it was not generally applied. Klebs and Brefeld's gelatine-methods of preparing culture media were already in operation; and the introduction, in 1881, of Koch's methods of isolation by means of plate-cultures simplified the preparation of pure cultures and afforded further facilities for bacteriological research. Koch's dicta, in 1883, established the recognized procedure necessary for the definite determination of a disease due to a specific organism: viz. (1) it is essential that the organism be present in the diseased tissues; (2) that it be grown artificially in suitable media for several successive generations; (3) that inoculations from the pure cultures so obtained should produce the same manifestations of the disease in healthy tissues, and (4) the same organism must be again isolated from the artificially infected tissues.

In view of such advances and after the impetus given to Animal Pathology by the researches of Koch and Pasteur, it appears strange that more attention was not devoted to the part played by Bacteria pathogenic to plants. Very few workers were attracted to this field of research, the whole principle of bacterial plant-diseases met with doubt and opposition, and even later, when careful investigation and exact study of the life history left nothing to be desired, great reluctance was still shown to admit the truth of these conclusions. In 1882 Dr. R. Hartig stated his conviction that there was no such thing as diseases of plants due to Bacteria. In 1884 de Bary asserts that they have scarcely ever been observed, and again in 1885 in the "Lectures on Bacteria" he assumes that present knowledge justifies him in regarding "parasitic bacteria as of but little importance as the contagia of plant-diseases." The whole subject is dismissed in some two pages with the mention of Wakker's Hyacinth, Burril's Pear and Apple Blight, Prillieux's Wheat disease, and Whemer's Wet Rot of Potatoes; and while admitting that saprophytic bacteria may, under special conditions, attack the tissues of living plants as facultative parasites, he concludes by a repetition of the statement that Bacteria are not objects of great importance in diseases affecting plants.

Hartig considered the plant-organism protected from bacterial intrusion, owing to its peculiar structure and the absence of circulatory channels which would serve for the distribution of micro-organisms, and that serious obstacles to their passage were presented by the impervious character of the non-nitrogenous cell-walls. Further, that the acid reaction of the cell-sap operates unfavourably for their growth. This latter view was also shared by de Bary. The reasons advanced by Hartig are merely theoretical, and when submitted to actual experiment have been shown to break down. The citation of recent work upon the secretion of a cytase by bacteria and their penetration through the softened cell-wall, that showing the entrance of the bacteria through the water-pores and their power of living and travelling in the xylem vessels, is sufficient to indicate how completely his conception was at fault. Though the influence of the acidity of the cell-sap has an important bearing upon the tendency to disease, it is now well known that the nature of the cell-sap offers no absolute resistance to the active growth of bacteria. It has been proved that the reaction of the parenchymatous tissues is by no means always acid, and moreover certain bacteria have been found to flourish best in distinctly acid media; while others possess the property of producing alkaline secretions which assist their penetration into the cells.

It is possible that the authority of such names as those of de Bary and Hartig may have had a deterrent effect upon the study of this branch of plant pathology. Nevertheless, a mass of literature gradually accumulated in favour of bacterial parasitism. In 1896 E. F. Smith published, in the American Naturalist, " A Critical Review of the present state of our knowledge upon the Bacterial Diseases of Plants." He drew attention to the unsatisfactory nature of much of the work in this field, and the need for full descriptions of the various forms, including a study of both morphological and biological peculiarities. At the same time he emphasized the importance of the strictest cultural technicalities and rigid tests of pathogenesis, which have too often been disregarded. His review of thoroughly investigated examples up to that date leaves no doubt that certain well-marked plant diseases owe their origin solely to a specific parasitic bacterium.

The accepted evidence up to 1897 rested upon much carefully conducted work, based upon Koch's four premises, in which the organism had been studied in pure culture, and repeated inoculations from pure cultures produced always the characteristic pathogenic symptoms and the reappearance in the tissues of the plant of the same specific organism. The diseases thus conclusively established may be briefly summarized:—

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| Pear-blight—*Bacillus amylovorus.* | Burril | 1880. |
| | Arthur | 1884. |
| | Waite | 1895. |
| Yellow Disease of Hyacinth—*Pseudomonas hyacinthi.* | Wakker | 1883-1889. |

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| Canker of the Olive—*Bacillus oleæ.* | Archangeli | 1886. |
| | Savastano | 1886. |
| | Prillieux | 1890. |
| Corn-blight—*Bacillus zeæ.* | Burril | 1889. |
| Potato Wet-rot—*Bacillus solaniperda.* | Kramer | 1890-1891. |
| Soft Rot of Hyacinth—*Bacillus hyacinthi-septicus.* | Heinz | 1889. |
| | Smith | 1897. |
| Bacteriosis of the Vine—*Bacillus uvæ.* | Macchiati | 1894. |
| Cucurbit Wilt—*B. tracheiphilus.* | Smith | 1895. |
| Brown Rot of Cruciferæ—*Pseudomonas campestris.* | Smith | 1896. |
| Potato and Tomato Disease—*Bacillus solanacearum.* | Smith | 1896. |

Migula, in his System der Bakterien, May, 1897, although still considering that the impenetrable cell-wall of plants presents great difficulties to the entrance of bacteria and that stomatal infection is generally impossible, yet allows that these objections do not universally hold. He admits that a number of bacterial diseases have been established, and devotes considerable space to the discussion of many well-known cases. Migula's attitude upon this question is in great contrast to that of Dr. Alfred Fischer at the same date. In the "Vorlesungen über Bakterien" (July, 1897) Fischer, in spite of the evidence available, expressed complete disbelief in the existence of bacterial diseases of plants. With the exception of the root-nodules of Leguminosæ, he professed to know of no single instance where bacteria invade the closed, living cells of plants, and states that the uninjured plant is "quite impregnable to their attacks." He maintained that bacteria live metatrophically only in diseased plant tissues "that have already been disintegrated and decayed by parasitic fungi." That the bacteria may "assist these subsequently in their work of destruction and modify perhaps more or less the character of the disease, but except for injuries from frost or insects the first attack on the plant is always made by fungi. All the cases of so-called bacteriosis of plants from the 'gommosæ bacilliare' of the Vine down to the 'schorf' of the potato, are primarily diseases of non-bacterial origin in which the bacteria are present merely as accidental invaders." He even goes so far as to state that "infected wounds are dangers that have no existence for plants," owing to the development of wound-cork which would cut off the provision of moisture and supplies of nutriment to the exclusion of the further progress of any pathogenic bacteria. As will be seen later, the rapid destruction of the cells, due to the activity of a bacterial parasite, as a rule precludes this protective tissue being formed; and the idea that fungi are always responsible for the primary attack is not in accordance with the cases described in which no trace of a fungal hypha was present. It is not possible here to enter in detail into a discussion of the points at issue, but Fischer's whole conception of the case showed such ludicrous ignoring of demonstrated facts in bacteriological research and such retrograde notions of the general physiological aspects of microbial infection, that some refutation was necessary. E. F. Smith, to whose investigations in this branch of plant-pathology we owe so much, took up the challenge and had no difficulty in showing the completely erroneous nature of Fischer's statements and "unwarranted assumptions." Smith has proved that in the case of the "Black-rot of the Cabbage" fully 90 per cent. of the infections take place through the water-pores, which provide in the epithem-tissue all the elements in solution necessary for the growth of bacteria. This ready entrance effected by *P. campestris* through the water pores has also been confirmed by H. L. Russell. As shown by Gardiner the water glands are continuous with the termination of a fibrovascular bundle, which thus furnishes a readily accessible channel for the progress of the attack. Stomatal infection has also been observed by Smith in a disease of Japanese plums, caused by *Pseudomonas pruni.* Waite proved by his experiments on Pear Blight that a large proportion of the infections take place naturally by means of the floral nectaries. The stigma is another part of the plant which presents an unprotected mode of access, and Kissling's work on the biology of *Botrytis cinerea* supplies an instance of very facile infection of the gentian through the anthers and stigmatic surfaces.

It would have been unnecessary to allude to Fischer's theories and mis-statements, were it not for the fact that in the English translation of his Lectures, issued by the Clarendon Press in 1900, the same errors are reiterated. This is all the more striking as this translation was published under the author's sanction and enjoyed the advantage of a proof-revision by Marshall Ward. Ward in general held the view that Bacteria in association with plant diseases were but a secondary accompaniment of the malady, and in his treatise upon "Disease in Plants" this author makes no allusion to the destruction of cellulose by bacteria, which plays such an important rôle in the penetration of the cells and the rapid disintegration of living vegetable tissues.

As early as 1850 Mitscherlich announced to the Academy of Berlin his discovery of the fermentation of cellulose, which he demonstrated by experiments upon the cells of the potato. His material contained no trace of any fungus, and he suggested that the "vibriones" which were present in great abundance must be the agents responsible for the phenomenon. In 1865, Trecul, in the course of his researches upon laticiferous vessels, observed the appearance of minute bodies in the tissues under examination which seemed to him to arise quite suddenly and spontaneously in the laticiferous vessels and closed cells. These bodies, which he termed *Amylobacter*, furnished him with an argument in favour of spontaneous generation, only disposed of when, later, van Tieghem showed them to be stages in the development of a Bacillus, named by him *B. Amylobacter*, and identical with the "vibriones" which Mitscherlich had rightly supposed to be the active agents in the dissolution of cellulose. van Tieghem clearly showed that certain Bacteria possess the remarkable property of dissolving cellulose, but he was undoubtedly working with mixed cultures and was mistaken in attributing his results specifically to *B. Amylobacter*. Prillieux's observations upon the breaking down of the cell-walls under the action of *B. tritici* have already been mentioned. Van Senus, in 1890, attributed the fermentation of cellulose to a cellulose-dissolving enzyme produced by the symbiotic action of two bacteria, one aerobic and the other anaerobic. Later, Omeliansky (1895) isolated, from the mud of the river Neva, an anaerobic bacillus which entirely dissolved filter paper with the greatest rapidity. These investigations, however, dealt with organisms acting saprophytically. The question of the destruction of the cell-wall of living plants by the action of parasitic bacteria was first definitely established by the researches, published simultaneously, of Laurent and myself (1899).

Laurent, in his investigations upon the potato and the causes of its greater or less resistance to bacterial disease, established the existence of a cytase, which dissolved the middle lamella, rapidly softened the cell-tissues, and caused the disaggregation of the cells. The organism which was the chief subject of Laurent's researches, *B. coli communis*, is very rarely capable of living as a parasite upon potato tubers and other plants. It was necessary for the tubers to be deprived of resistance, by means of exceptional cultures, to enable the bacillus to develop upon the potato. From that point its virulence was increased by successive cultivations upon tubers of slight resistance, until varieties at first highly resistant ended by becoming invaded by the parasite. The virulence disappeared as soon as the microbe ceased to be cultivated on a living tuber, cultures in nutritive solutions served to suppress the aptitude of the parasite, and henceforward it could only be restored after special preparation in alkaline solutions. In this he demonstrated a complete parallel with Kissling's researches on *Botrytis*.

The bacterium, causing the "white-rot" of turnips, which was the subject of my special research, belongs to the genus

*Pseudomonas* and illustrates a very virulent form of parasite. It was isolated from turnips attacked in the fields and, unlike *B. coli communis*, flourished on nutritive media, and even after many cultivations could readily be inoculated from these on to pieces of living turnip, producing all the effects of the white rot in about 12 hours. This organism was grown in pure culture from a single bacterium and, both when living in a nutrient solution and on a living turnip, was found to secrete an enzyme which has the power of dissolving the middle lamella and of causing the softening and swelling of the cell-wall. I also demonstrated the production of oxalic acid by the bacterium, and that this acid acts as a toxin in plasmolysing and killing the protoplasm. This proof of the secretion of a cellulose-dissolving enzyme introduced a new factor, and finally disposed of the " impassable barrier " supposed to be offered by the cellulose membrane to the entrance of bacteria. As the result of further researches I was able to trace, by continuous observation, the actual penetration of the bacterium through the cell-wall. The observation of the movements of the bacteria, though difficult and very trying, was yet considerably furthered by the difference of refractive index between the cell-wall and the bacteria, which enabled the course of the latter to be distinctly followed.

It is not until the protoplasm has been killed by the toxin and the cell-wall very much softened by the cytase that the bacteria have the power of perforating the walls and passing into the cell-cavity. It would hardly be supposed that a single bacterium, through its own exertions, could soften the cell-wall and pierce it at one definite point after the manner of a fungus germ-tube. The extreme minuteness of the bacteria and the rapidity of their multiplication lead them to act, as it were, in concert, and the wall becomes softened by the cumulative action of many bacteria before the penetration of a single individual.

The old and fully developed cuticle is apparently proof against the action of the enzymes excreted by *P. destructans*, but this parasite can readily effect an entrance into its host through the undeveloped epidermis of young and tender structures. It is incapable of manipulating the hard and tough rind of the sound turnip, but when brought into contact with a wounded surface it at once flourishes as a saprophyte upon the remains of the injured cells; very soon the number of bacteria becomes largely increased, and the toxin and cytase have sufficiently accumulated to kill the first cell. With the death of its protoplasm the cell-contents are liberated, and an additional supply of nutriment is thus provided; the bacteria continue to multiply, cytase and toxin continue to be set free, and thus each cell succumbs in turn.

A comparison of the parasitism of *Botrytis cinerea*, as demonstrated by the investigations of Nordhausen, presents an exact parallel. He has shown that the spore of this fungus excretes a powerful toxin in its initial stages of germination, before any trace of the germ-tube can be detected. Its manner of effecting an entrance into a host-plant is first to kill the cell by the emission of the toxin; the germ tube then penetrates the dead cell and is nourished saprophytically upon it; with the vigour thus gained it destroys the neighbouring cells and passes from one to another without difficulty. The fungus hypha has the power of perforating the cuticle, but only in young and tender structures; old and hardened membranes could only be entered when the cuticle had been injured, or when it had gained strength by special saprophytic nutrition. Thus the bacilli play here a part absolutely comparable to that of the fungi and a complete homology is established between them.

This form of parasitism is apparently typical of a large class of bacterial diseases, in which there is a rapid degeneration of the cell-wall and complete destruction of the parenchymatous tissues. Van Hall's researches in 1902 demonstrated the action of a toxin secreted by *Bacillus omnivorus* when attacking *Iris florentina*, and four years later (1906) Harrison isolated a cytase in the case of a disease of cauliflowers caused by *B. oleraceæ*, which exhibited symptoms identical with those produced by *Pseudomonas destructans*. A cytase is also concerned in the rot of the Carrot and other allied plants, described by Jones (1905); and probably in certain potato rots, though in these last instances the enzyme has not been isolated. Another species of *Pseudomonas* producing a brown rot of the Turnip, which I have had under investigation, belongs to a group working in a totally different manner; the action is very much slower, and the rapid swelling of the cell-wall is not a conspicuous feature. The tumourous diseases, such as the canker-knots of the Olive, caused by *Bacillus oleæ*, also exhibit a comparatively slow development.

Another type of bacterial disease is that in which the xylem vessels are primarily attacked and become filled with numerous bacteria. As a consequence the transpiration current is unable to flow along these channels, the supply of water is cut off, and hence a withering of the shoots occurs. In the bacterial disease of Sweet-corn, described by F. C. Stewart, the organism is confined exclusively to the fibro-vascular bundles and never pervades the cells of the parenchyma. In many other cases there is subsequent invasion of the parenchymatous tissues, and total destruction of the cell and cell-contents ensues. Examples of this type are found in the wilting of various Cucurbitaceæ traced to *B. tracheiphilus;* the bacterial disease of the Tomato, Egg-plant, and Irish Potato; the Yellow-rot of Hyacinths; the

Bacteriosis of *Dactylis glomerata*, etc. In this category must also be included the Brown or Black rot so prevalent in species of the genus *Brassica* and other Cruciferæ. A striking symptom of this disease is the blocking of the lumen of the wood-vessels and also the neighbouring intercellular spaces with a kind of gum or mucilage. This gum is most insoluble. It stains red with phloroglucin and reacts to thallin sulphate, but remains uncoloured under the phenol-potassium-chlorate hydrochloric acid test, thus bringing it within the vanillin group. It is a substance probably derived from the soluble carbohydrates. There are many "gum-diseases," such as the Gummosis of the Beet-root, Sugar-cane, and Vine, and the gummy flux of the Amygdaleæ, which have now been traced to the activity of certain definite Bacteria.

It is impossible to do more than briefly indicate the characteristic features of attack in a few cases; it will be understood that numerous other examples have been worked out which have been obliged to be left quite unrecorded.

In addition to their rôle of parasites, the Bacteria function very actively as saprophytes, following in the wake of parasitic fungi and completing their work of destruction, and this observation has no doubt led to the scepticism as to their aptitude as true parasites. Thus Ward (1898) states: "Without going so far as to say there is no bacterial disease of the potato, I wish to express the conviction that the alleged cases of such lately published are not convincing, and that a tendency exists to draw conclusions from imperfect evidence. I shall show that the way into the tuber is prepared for bacteria by fungus hyphæ and the open passages of destroyed vascular bundles affords them ample space." No one would seriously think of contesting this last statement; that bacteria should be present in many fungoid plant diseases is but natural to expect, and that their part is only secondary may be true in certain cases, but undoubtedly the converse is also true, that is, that the bacteria are often the prime agents in paving the way for the fungus hyphæ. In cases where bacteria and a fungus are associated together in a plant disease, it is necessary to isolate these organisms and grow them in pure culture. As regards the bacteria, modern methods of culture have rendered this a fairly easy task, but to obtain the fungus free from any bacteria is a matter of the utmost difficulty. The definition of a pure culture in the case of fungi must be extended. It can no longer mean that no other fungus is present, but must include the conception that bacteria are also entirely absent.

A promising field of enquiry awaits the investigator into the relation between bacteria and fungoid parasites, and their association one with another in plant pathology. In the disease known as "Finger and Toe," bacteria are always present in conjunction with *Plasmodiophora*, and hitherto, I believe, a culture of *Plasmodiophora* free from bacteria is unknown. Pinoy considers that they play an active part in this disease. The parasitism of *Fusarium* affords a further illustration. A species of *Fusarium* commonly met with on Turnips and Swedes in Northumberland, is apparently responsible for a large number of diseased roots in the fields. But in the decaying roots bacteria in abundance are invariably associated with the hyphæ, and so far, considerable difficulty has been experienced in obtaining a culture of this fungus entirely free from the bacteria. Until this has been accomplished the question of its parasitic nature cannot be decided in this case. Wehmer and Frank claim to have grown *Fusarium solani*, from a pure culture, as a parasite upon the potato; but these and other published accounts still leave a doubt as to the absolute exclusion of bacteria throughout the entire experiments. Under natural conditions of infection at least, it remains an open question whether the bacteria or the *Fusarium* is the secondary factor or whether the destruction of the host-cells is due to their combined influence. In the Erysiphaceæ again one would certainly expect innumerable bacteria to be present on the leaves together with the fungal hyphæ, but nothing is known as to their action, and apparently these organisms have been entirely left out of consideration here, as in many other fungoid parasites.

The epiphyllous lichen flora is a striking feature of the tropical rain forests, and one is naturally led to search for its analogue in temperate climates. Burri (1903) has shown that an actively living bacterial flora is ordinarily to be found on leaves, and that these bacteria form a special class quite distinct from those normally present in the air or soil. The number of bacteria actively existent upon the surface of leaves may be several millions per gram. of leaf, while the number of those in the resting condition (presumably accidentally deposited) is always relatively very few. No relationship could be established between the number of bacteria and the atmospheric conditions. Düggeli has also shown that certain bacteria accompany dry seeds or fruits, and on germination find their way upon the leaf-surface.

In addition to the well-known epiphyllous fungus *Apiosporium (Fumago)*, I have found that other fungoid and bacterial germs are extensively present upon the surface of healthy leaves under the ordinary conditions. This was strikingly exemplified by impression cultures of leaves made upon the surface of a nutrient gelatine, in petri capsules. While still attached to the plant the leaves were lightly pressed upon the gelatine in the capsule, which was only momentarily opened for this purpose, and an impression of the leaf surface was thus obtained. In every case incubation after 2-3 days showed numerous colonies of bacteria; and fungi, chiefly represented by species of *Penicillium* and *Botrytis*, were also met with. The colonies were confined to the area of the leaf impression, which was distinctly outlined in this way, and no growths appeared on the surrounding medium (Plate 10). The organisms were equally abundant on both upper and lower surfaces of the leaf, and the species apparently vary with the season and the kind of leaf.

Since this epiphyllous flora is always present upon the surface of green plants, it becomes a matter of considerable interest to determine the part played by these micro-organisms in the ordinary fungoid diseases. Are these bacteria at all concerned in the problem of immunity? Do they in any way modify the life-histories of other bacteria or fungi with which they come in contact? I would merely throw out a suggestion that without being in any way harmful germs, they may yet profoundly influence existing conditions in some unsuspected way.

The question whether non-pathogenic micro-organisms are normally present in plant tissues and can maintain their existence in the intercellular spaces is another interesting speculation. It must be remembered that for the purposes of respiration, etc., the intercellular system of plants is in constant communication with the surrounding atmosphere, and thus an easy entrance is afforded through the stomata or lenticels. Whether having gained an entrance the bacteria can actively live, or persist as spores, in the intercellular spaces requires further elucidation. In 1887 Gallipe's experiments led him to the conclusion that the soil micro-organisms enter the roots or tubers of many plants, and in 1888 Bernheim announced that micro-organisms are to be found in the Indian corn and other cereal grains. These conclusions have been very adversely criticized, and neither Buchner, Lehmann, nor Fernbach and Di Vestea have been able to confirm these results. Lominsky, however, finds that the soil bacteria can pass into the root tissues; and Fernbach and Di Vestea, though considering the interior of healthy, uninjured tissues to be free from bacteria, yet grant the fact of their presence in the interior of cut plants exposed to a damp atmosphere.

The method employed by the investigators mentioned was generally to sterilize the surface of the plant tissue in the bunsen flame, to remove a small portion of the uncharred tissue beneath with a hot scalpel, and immediately place it in a sterile nutrient medium. Following up this method I selected various roots or tubers taken straight from the ground, such as Potatoes, Swedes, Turnips, Carrots and Beetroots. These were cut with a heated knife, the cut surface thoroughly charred upon an iron plate placed over a bunsen flame, and small pieces from the interior removed with a heated scalpel and placed in sterile plugged test-tubes. Only in some cases were Bacteria found to develop. When, however, the roots or tubers were first kept in the laboratory for 3 days in a damp atmosphere and then treated in precisely the same manner Bacteria were always found to be present in the tissues. As far as the evidence goes it appears to indicate a possibility that the intercellular spaces of storage organs in the natural state may harbour living bacteria, but that they would almost certainly be present in detached portions of plants subjected to a damp atmosphere or other abnormal conditions.

Pasteur has determined that bacteria are not present in the normal healthy animal tissues. This view is also generally held with regard to vegetable tissues, but some more conclusive experiments are needed to decide this point in the case of plants.

In many physiological experiments connected with plants, the existence of bacteria, both on the external surface and possibly in the intercellular spaces, is ignored, but the action of the various micro-organisms present must have contributed in some measure to the effects recorded. That a neglect of such considerations may lead to serious misconception is exemplified by the observations of Stoklasa. The generally accepted view that an injured plant organism breathes more actively than an uninjured one is shown by him to be incorrect. The experiments by Stich were not conducted under conditions free from bacteria, and when repeated by Stoklasa under sterile conditions were found to give opposite results. Under proper precautions the respiratory activity of injured cells proved to be less than that of uninjured tissue, and the increased production of $CO_2$ at a wound was traced to the activity of the Bacteria living upon the injured cells.

The external conditions to which any plant is exposed have an important bearing upon its general health, and render it more resistent or more susceptible to parasitic attack. *Phytophthora infestans* may be cited as a familiar instance. It is generally recognized that light, and the temperature and vapour pressure of the air, influence in a marked degree the destructive action of this fungus and presumably also of other fungoid and bacterial parasites. Again the temperature, air and moisture-content of the soil, and the nature of its food constituents, are all forces necessarily affecting the general vigour of any host-plant. There is considerable evidence that susceptibility to disease is influenced by manurial treatment and that abundant fertilization, especially with nitrogenous manures, renders the host less resistant to microbial invasion. Laurent has shown how the susceptibility of a given variety of potato was related to the manurial treatment under which it was cultivated. Thus the variety Simpson, when grown in a soil manured with phosphorus, maintained a high degree of resistance to certain bacteria, which was totally lost when grown on the same soil liberally manured with lime. This he attributed to the action of the lime in liberating ammonia.

The direct relation of the character of the cell-sap to the question of immunity is also a well ascertained fact, though Laurent proved that the *total* acidity bore no relation to the degree of resistance. Experiments showed that the resistance of tubers of potato to bacterial invasion was due to soluble substances which exist in the cell-sap, and that the immunity could be destroyed by subjection to alkaline solutions. The effect which a difference in chemical composition of the plant-tissues can exercise upon the development of a virulent form of parasitism is strikingly exemplified by Laurent's experiments with different forms of *B. coli communis*, *B. fluorescens*, *B. enteridis*, *B. typhique*, etc. All of these species were found capable of living as true parasites on the potato after special treatment had first diminished the resistance of the cells, the typhus bacillus showing the most surprising results in power of virulence.

May we not consider that the different forms of *Botrytis* raised by Kissling, as also the different forms of *B. coli communis* of Laurent, are "biologic forms" and that the foundation of this theory was laid by Kissling in his work upon *Botrytis*.

The past few years have been remarkable in considerably extending our knowledge of parasitic diseases and in opening out new avenues of research. The parasitism of bacteria has been established equally with that of the fungi and much confusion has been cleared away. But the mutual relationship of these parasites in certain plant diseases still demands attention, and their action upon the living cell requires much further elucidation. With a knowledge of the fact that nutrition may so alter a facultative saprophyte that it becomes a virulent parasite, while through other nutritional changes its virulence may be entirely lost, and further that the same influence operates in rendering the host more or less susceptible, we have the key to one of the important determining factors in the epidemic diseases of plants. It is no longer sufficient to trace life-histories or to prove parasitism under certain special conditions. The nutrition of both host and parasite must be taken into account, together with other factors which tend to disturb the nice balance existing between conditions of health and disease. We must look to the application of the laws of physical chemistry, in the botanical laboratory and in the field, for a solution of these and other problems connected with Plant Pathology.

## LITERATURE.


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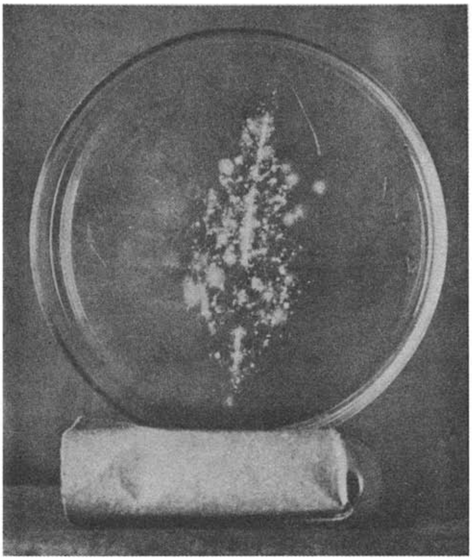
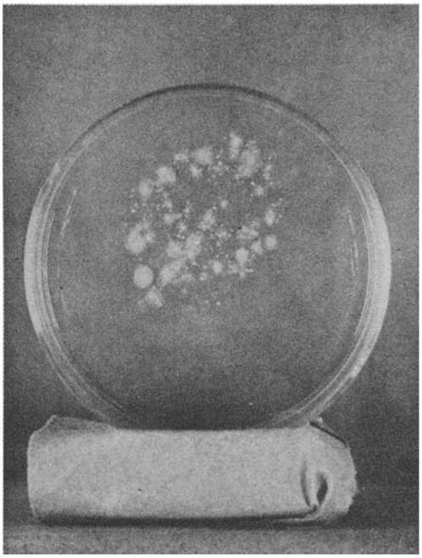
## Description of Plate 10.

Impression cultures of leaves upon nutrient gelatine, incubated for three days (Sep. 4-7), showing the colonies developed. A, upper surface of the leaf of an artichoke *(Helianthus tuberosus)*; B, under surface of the leaflet of a potato *(Solanum tuberosum)*.

A.





B.

West, Newman proc.