MICROBES

IN

FERMENTATION, PUTREFACTION,

AND

DISEASE.

PAPER READ BEFORE THE PHILOSOPHICAL SOCIETY OF GLASGOW,
DECEMBER 14TH, 1881.

BY

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LONDON:
BAILLIERE, TINDALL, AND COX.

1882.

INTRODUCTION.

The following Paper was read before the Glasgow Philosophical Society in December last, and appears in the Volume of Transactions of the Society, recently published. In that volume it chanced to attract the attention of Professor Tyndall, who wrote me thus:

> "In the undisturbed quiet of this day—Sunday, the "29th of October—I have read your article in the Proceedings of the Philosophical Society of Glasgow, 'On "Microbes in Fermentation, Putrefaction, and Disease.' "Will you permit me to thank you for the pleasure I "have derived from the perusal of that article. Matthew "Arnold himself could not find fault with its 'lucidity,' "while, as regards knowledge and grasp of the subject, "I have rarely met its equal."

Such a testimony to the merits of the Paper, coming spontaneously from the highest authority among English speaking men on the branches of science to which it relates, a gentleman, too, with whom I could claim no personal acquaintance, could not fail to be peculiarly gratifying. Although desirous of bringing before the medical and scientific world the views contained in the paper, I had taken no steps for that purpose chiefly from a conviction that without the imprimatur of some weightier name than mine they would receive no attention. This deficiency Professor Tyndall's letter abundantly supplied, and explaining my motive for doing so, I asked his permission to make public use of it. To my request he most courteously acceded, and hence this preface—hence this reprint of my paper.

CHARLES CAMERON.

November, 1882.

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MICROBE is a word with which the recent researches of M. Pasteur into the intimate pathology of fowl cholera and splenic fever have made the public of this country more or less familiar. It was originally introduced by Sedillot as a convenient and non-contentious name for various minute organisms found in the fluids or tissues of the body in certain diseased conditions. As generally employed, its use is confined to them. As we shall see, however, that precisely similar organisms are inseparably connected with the processes of fermentation and putrefaction, and in certain cases that specific organisms associated with these processes, when they find their way into the animal economy, give rise to specific diseases, I see no reason why the use of the word should be so restricted, and shall apply it indifferently throughout this paper to the minute organisms which play such a prominent part in the whole three classes of phenomena. Though these microbes are generally believed to belong to the vegetable world, in lower regions of life the boundaries between the animal and vegetable are very indistinct, and there are many microbes which display so much activity and such an apparent intelligence in the direction of their movements, that it is difficult not to share the sentimental preference to which Pasteur gives expression for placing them in the same kingdom as ourselves. They consist of cells of different sizes and shapes, which multiply themselves with enormous rapidity and are characterised by a considerable resistance to various reagents, such as ammonia, concentrated acetic acid, &c., which destroy animal cells, a characteristic of which the microscopist avails himself in his demonstrations when he has to deal with them in animal tissues. They have been elaborately classified by certain botanists; but experience shows that in many cases they vary in form according to the medium in which they are found, and some of them possessing the most distinctive properties, like the fermenting and non-fermenting torulæ, for example, are undistinguishable under the miscroscope.

As my principal aim is to call attention to recent researches regarding their connection with disease, under circumstances where their vital reactions are of supreme importance, I shall not waste time in attempting any description of their morphological differences, and shall content myself with roughly indicating the general characteristics of some of the species of organisms to which I shall most frequently have occasion to refer. I shall have to speak of the *torula* or yeast plant, a spherical or ovoid organism of which there are various species, varying very much in size; the *micrococcus*, a family of which there are also innumerable species, presenting the most diverse characters—sometimes found in the form of separate spherical cells; sometimes of chains or bunches of cells held together by a gelatinous bond; sometimes gliding over the field of the microscope by a combined sinuous movement; sometimes jerking upon their bonds of union with a rythmical movement which has been compared to the brandishing of a shillelagh. Again, we have the *bacterium* —a rod-like group of microbe—of which some species are hundreds of times larger than others; the *vibrio*, an elongated cell, often manifesting the most lively movements, swimming about like a tiny eel, and presenting itself in as many shapes as a leech. Of all these groups there are may species, some of which have been carefully studied and identified, but the vast mass of which are as yet to be described and classified.

Now, I need not tell you that of late years these microbes have been shown to play a most important part in many diseases; but their characteristics naturally admit of much more accurate and exhaustive study in the processes of fermentation and putrefaction than when they are complicated and obscured by the disturbing reactions of concurrent life. If, therefore, we wish to understand their functions in disease, we must, in the first place, consider what is known regarding them in the other processes in which they intervene. I accordingly propose to direct your attention to certain points in connection with their intervention in fermentation and putrefaction, which seem to be essential to anything like a clear understanding of their effects in disease. And if I seem to dilate at too great length upon this branch of my subject, my apology must be, that in this country the purely chemical teachings of Liebig regarding fermentation have left so deep an impression that, even to this moment, they cause the vast majority of us to overlook the importance of the antagonistic and vital theory of Pasteur—a theory which has directly led that scientist up to the wonderful results with which the world has recently been startled into amaze.

The amount of pure yeast which can be taken upon a pin's point will suffice to accomplish the fermentation of an unlimited quantity of wort. The reason is that the yeast cell is a living plant, and grows so long as it finds material to feed on, and its growth under proper circumstances is accompanied by the decomposition of the sugar contained in the wort into alcohol and carbonic acid. The life of the plant and the decomposition of the sugar are cause and effect. Boil the yeast and no fermentation will result on its introduction. Destroy its cells by rubbing them on a ground glass plate and the same thing results. Retard its growth by cold, and the decomposition of the sugar is retarded; accelerate it by heat, and the production of alcohol is accelerated; kill it by compressed oxygen or in any other manner, and the fermentation stops. But the yeast plant is far from the only plant which is capable of giving rise to this decomposition, and under certain circumstances it fails to give rise to it. In the first place, there are a number of yeasts, doubtless descended from some common ancestor but presenting specific differences, giving rise to slightly differing products, and incapable of transformation one into the other. The ordinary yeast plants of our breweries produce spores only under extraordinary circumstances of artificial cultivation, but the microbes which give rise to the alcoholic fermentation of the bruised grape are produced by spores which are self-sown, which develop ferment cells only at certain seasons, and whose origin is to a large extent shrouded in mystery. Various moulds, submerged beneath the surface of the saccharine fluid in which they vegetate, and so deprived of the oxygen which, under normal circumstances, they derive from the air, continue to live, and extracting that oxygen from the sugar amid which they are plunged, give rise to the formation of alcohol and carbonic acid. In forming the sugar which abounds in ripened fruits, the unripe fruit absorbs oxygen from the air. If the ripening fruit be placed in an atmosphere of carbonic acid, cell life continues to go on in its interior, but the process is reversed. The cells appropriate the oxygen, of which they have need, from the sugar already formed around them, and the production of alcohol and carbonic acid again results. Even in fresh meat, where organic cell-life continues feebly long after the animal from which the meat was cut has perished, Bechamp has found alcohol—doubtless the result of an analogous vital process. Nay, within the present year

M. Muntz, by a delicate reaction, has shown that alcohol, in minute quantities, is to be found everywhere in nature—a result of the decomposition of organic matter by the ferments which everywhere exist. What is the explanation of all this? I can conceive none other satisfactory than that given by Pasteur years before many of the facts I have mentioned were known. "The character of ferment," he says, "presents itself to us not as proper to such and such an "organism or such and such an organ, but as a property of "the living cell, a character always ready to manifest itself, "and really manifesting itself, as soon as life is not accom-"plished under the influence of free oxygen gas, or of a "quantity of this gas insufficient for all the acts of nutri-"tion. One sees it commence and cease with this species of "life—slight and of feeble duration if the species of life is "the same; intense, on the contrary, and of long duration, "furnishing large quantities of alcohol and carbonic acid, "when the plant, the organ, or the cell can multiply itself "with facility under the new conditions. Thence all "imaginable degrees of fermentation, and thence also the "existence of ferments of all forms and of every different "species. One can even imagine without difficulty," he goes on to say, "that the decomposition of sugar should "be altogether different from that of which we have spoken, "and that, in place of alcohol and carbonic acid, it should "give glycerine, &c., or lactic, butyric, acetic acid, &c. This "would occur with a certain class of cellular vegetables "only, more or less analogous to those which decompose "sugar into alcohol and carbonic acid. Others specifically "different would act in a different manner. In a word, so "many beings, so many different ferments." Fermentation of this class is then a process inseparably connected with the functions of life. In certain cases, as in ordinary acetic fermentation, the microbe takes the oxygen which it requires from the air, and with it burns the combustible portion of the nutriment it assimilates, giving off carbonic acid, and leaving behind a compound of proportionately higher oxidation. In another class of fermentation the microbe falls back upon the oxygen of the organic compounds in which it is found, appropriating that to the purposes of its own development and reducing them to a state less rich in oxygen. And the same organism may, under certain circumstances, act in one manner or the other. Thus the *microderma vini*, which in certain circumstances reduces sugar to alcohol, will, under others, oxidize alcohol into acetic acid. But, although there are certain of these organisms or microbes which accommodate themselves with great seeming indifference to either mode of life, there are others essentially aerobic or anaerobic—for which free oxygen is a necessity or a poison—which can apparently be more or less successfully trained into tolerating or dispensing with it under certain conditions of development and cultivation, but which, under the circumstances in which we are ordinarily acquainted with them, display of their energies only in one direction and in a specific manner. Thus the *torula cerevesiæ* in the everyday development of its life resolves sugar into alcohol. An organism very similar in appearance, but much smaller, produces lactic acid. A third, the *microderma aceti*, oxidizes alcohol into acetic acid. A fourth, a lively vibrio, reduces fermentable matter to butyric acid with the liberation of hydrogen, which, when the matter fermenting contains phosphorus or sulphur, unites with them and produces the unsavoury odours characteristic of putrefaction. Another organism again—a little micrococcus—decomposes urea into ammonia, and hippuric acid into benzoic acid and glycocole. The transformation of tannic acid into gallic is the work of a sixth. The artificial conversion of ammoniacal waters into nitre appears to be due to the intervention of a seventh. And I might mention as many more instances of specific fermentation without exhausting the list of those of which the specific microbe has been demonstrated or studied.

We are now in a position to turn to the process of putrefaction, and to understand precisely what that is. In a sentence, putrefaction is neither more nor less than a process of impure and mixed fermentation, the products of which vary according to the species of the ferments which happen to have been sown, the nature of the soil on which they find themselves, and the circumstances in which they are placed. A certain step in the reduction of organic matters to their elements is effected by one ferment. The new compound is taken up by a second, and advanced a stage further on the way to dissolution. That again is laid hold of by a third, until finally the organic structure is reduced to its original inorganic elements, and these are set free, once more to enter on a new cycle of life. A microbe which plays a leading *rôle* in this great work of reorganisation is the vibrio of butyric fermentation. To the fully-developed organism oxygen is fatal, and, when exposed under a microscope in a drop of liquid covered by glass, it is only in the centre of the field, where they are protected from air, that these vibrios retain life for any time. But their spores or germs are endued with the most robust vitality. They are to be found everywhere, and carried by the air into putrescible matter, they do their work in the lower strata, protected against the deadly influence of the atmospheric oxygen by the air-loving bacteria and microbes which occupy the upper surface, and monopolise the oxygen it dissolves, and in turn supplying them with butyric acid, which they, with the aid of the oxygen of the air, reduce by a slow combustion to carbonic acid and water. It is a fact, probably worth noticing in passing, that, according to a recent investigator, M. Van Tieghen, this butyric microbe is one of the most widely-diffused in nature. It possesses the faculty of decomposing cellulose, and it, according to Van Tieghen, was the agent which in the marshes of the coal epochs, as in those to-day, was the great destroyer of vegetable organs, dissolving the envelopes of the cells, and preparing the way for their fossilisation—a theory supported by his discovery in the organs so fossilised of numberless specimens and fragments of this very microbe, and innumerable quantities of its spores. But to return to my text.

If putrefaction is simply a form of fermentation, why have I commenced by speaking of the processes as if they were distinct? For this reason, that whereas certain phenomena of microbe life have been discovered in the investigation of fermentations connected with our industries, other peculiarities of infinite practical importance have been most elaborately studied in connection with the question of putrefaction and the problem of so-called spontaneous generation. Now, I am far from inappreciative of the importance of Dr. Bastian's work in the field. He has brought to light a number of valuable facts, and the pertinacity and eloquence with which he has maintained his favourite theory has led to the invention of more rigorous methods of investigation, which have established the Harveyian maxim *omne vivum ab ovo* on a foundation infinitely solider than it ever stood on before. No one who has read the recent work of Professor Tyndall can doubt that the doctrine of spontaneous generation is dead, but in the investigations and discussions which have led to its death, knowledge of infinite value has been evoked. I need not tell an educated audience like this that it is now established beyond all contestation that, preserved from contact with microbes or their germs, the most putrescible bodies exposed for years to contact with the atmospheric air will remain unputrefied. I need not tell you that, under such circumstances, meat in contact with the air will undergo certain changes, but will not rot. Blood will disintegrate, its corpuscles will fall to pieces, and salts will crystallise out, but it will not putrefy. Wort will oxidize, but it will not ferment; and animal and vegetable infusions will remain for years without apparent change. But, in establishing these facts, it has been proved that the most extraordinary difference exists between the tenacity of life exhibited by the developed microbes and the spores or germs from which they spring. Submit the microbes to a boiling heat, or, in many instances, to a heat far short of the boiling point, and they are killed. Dry them, and in many cases they die at once, and in all in a comparatively short time. Expose certain of them to the oxygen of the air, and they perish. Saturate the fluid in which others are found with carbonic acid, and they are paralysed; and though for a time capable of revival by oxygen, ultimately succumb. Expose any of them to oxygen under high pressure, and they are asphyxiated. Dilute solutions of antiseptic agents kill them. But as to the spores which they produce, and from which succeeding generations spring, there is almost no killing them. The more you dry them the better they resist destruction. Time is no object with them, and they maintain their dormant vitality for an indefinite number of years. Absolute alcohol has no effect on them. As to oxygen, they can stand that concentrated by the pressure of twenty atmospheres, and be none the worse. Two or three hours' boiling, if they have been well dried beforehand, seems not to hurt them, and they have been even known to survive eight hours of the process. The only effectual means for their immediate destruction that I am aware of is the flame of a spirit lamp. To that their extreme minuteness renders them an easy prey. Now, how, you will ask, does it come, when these germs can stand so much rough handling without destruction, that mild solutions of such harmless antiseptics as carbolic acid or borax, or permanganate of potash, can have any effect in preserving us against the mischief they work? This strikes me as a most important point in connection with medicine and surgery, but I have never seen it noticed. Professor Tyndall's experiments, however, appear to afford a very simple explanation. That gentleman took infusions infected with spores or germs derived from old hay, on which many hours of continuous boiling had no effect, and found that by boiling it for a single minute, or fraction of a minute, at intervals of a few hours, he could, with an aggregate of five minutes' boiling, sterilise the most refractory infusion. The explanation is obvious. The spores in an infusion are in different conditions as to species, age, dessication, exposure to air and light, &c., and they will naturally require different periods for germination. While they remain simple spores they resist prolonged boiling. But allow them to germinate, and deal with each successive crop as it is springing into life, and your victory is of the easiest. In the same way we can easily see how any solution which will kill the developed microbe can preserve the decoction or wound in which it is placed from the development of microbes. It may not kill their refractory germs, but it will kill them off in detail as they spring into life. The point has a most evident bearing on the rationale of the disinfection of articles containing the germs of the microbes of disease, and I can't help thinking, urgently demands a much more stringent experimental investigation than it has hitherto received.

But what has all this talk about these microbes and their germs got to do with the subject of disease? In 1879, M. Marix made some experiments upon the action of ordinary yeast on blood outside the body. He found that it multiplied iteslf freely, depriving the blood corpuscles of their oxygen, and reducing them to a dark brown colour, and that it decomposed the sugar of the blood into carbonic acid and alcohol. He then tested its effect when introduced in suspension into the circulation of the living animal, and found that when more than two grammes of it was injected the result was always fatal, while in doses below two centigrammes, on the contrary, it was almost innocuous. Between these doses the effects observed were very characteristic. There was an elevation of temperature followed by a depression often extreme, and the animal became comatose. Almost immediately after the injection the subject of the experiment was seized with a diarrhœa strongly resembling that of typhoid fever, and after death the glands of the intestines, known as Peyer's patches, were found engorged and ulcerated precisely as in that disease. The blood was not altered in colour, showing that within the living body its corpuscles had sufficient energy to enable them successfully to contest that element with the yeast plant; but every trace of sugar had disappeared from it. In non-fatal cases there was the same fever, the same glairy and sanguinolent diarrhœa, in which the yeast globules were apparently eliminated, for after a few days they were not to be found in the blood.

When Pasteur discovered that the fermentation which changes urea into ammonia was due to the presence of a specific micrococcus, he ventured to assert that to the same microbe, and not to the decomposition of effused mucus, would be found to be due that formation of ammoniacal urine within the bladder which in cases of retention of that fluid gives rise to such distressing symptoms. This, subsequent observation has proved to be the case. Ammoniacal urine is as much the disease of the *micrococcus ureæ* as itch is the disease of a specific acarus.

Coming now to the ordinary microbes of putrefaction, we all know by what a remarkable process of analogical reasoning a former fellow-townsman of our own grasped the fact of the important part they play in surgical disease, and how—without more knowledge of their individual peculiarities than could be gained by a perusal of Pasteur's memoir on lactic fermentation—Lister laid the practical foundations of that great principle which has made him famous throughout the civilised world, and will give reason to countless generations to bless his name. The success which has attended his practice affords to the general public the most convincing proof of the correctness of his theory. This is not, however, sufficient for the man of science. But M. Chauveau, a veterinary surgeon of Lyons, who first demonstrated that infections were not liquid or gaseous but particulate, and whose many invaluable researches into the intimate phenomena of contagions are far too little known in this country—devised a demonstration which sets the matter beyond all question. For the production of wether mutton in France recourse is had to an operation known as bistournage. This consists of the rupture of the spermatic cords by torsion cutting off the testes from all communication with the circulation, but leaving surrounding tissues uninjured. In this operation the glands do not putrefy, but in time become adherent to the surrounding tissues, and undergo a species of fatty degeneration. This is what one would expect, for we have seen that without the intervention of microbes putrefaction will never occur. But Chauveau reasoned that if these were really the cause of putrefaction you should be able to produce it in the glands killed by the operation, though within the living body, by introducing these microbes into them through the circulation previous to its performance. He accordingly injected into the jugular vein of a sheep a quantity of fluid charged with microbes of putrefaction, which experience had taught him was insufficient to kill the animal, and then performed bistournage. Before he had done so the microbes had found their way into the testes and these putrified. But was the putrefaction produced by the microbes or by the fluid in which they had been conveyed into the circulation?

He filtered the liquid so as to separate the microbes, repeated the operation, and the result was *nil*. Finally, he performed bistournage on one testis so as to sever it from the circulation, injected the decomposing fluid, and then performed bistournage on the other side. The gland that had been cut off from the access of the microbes by bistournage previous to the infection of the animal remained sound; that into which they had been allowed to find their way previous to the operation rapidly putrefied.

I have described putrefaction as consisting of a varying mass of mixed fermentation, and if this theory is correct—and I am not aware that it is disputed—from what we now know of the action of individual ferments we should expect that the septic disease resulting from the introduction or absorption of putrefying matter into the system should be of a very complex nature; and so it turns out to be. Pasteur has described two principal forms of it, and the microbes giving rise to them in human beings; while Koch has identified no fewer than six upon lower animals, and it seems certain that the subject is not yet worked out. Koch's researches illustrate what is really an analysis of disease, and as the adoption of a method so analogous to that of the chemist promises great results to scientific medicine, I shall enter into it at some little length. Owing to the presence of so many ferment germs in the air it is impossible without the most extraordinary precaution, to obtain any one of them in a state of purity. The yeast of commerce is full of impurities, and can be made in a short time to produce lactic, acetic, or butyric fermentation. The superior vigour of the yeast plant prevents the microbes of these fermentations from doing much harm so long as alcoholic fermentation is going on with energy, but under certain circumstances they occasion great loss to our brewers. To separate different ferment microbes from each other you avail yourself of the fact that different species thrive best on different soils. One grows most vigorously in an acid infusion, another in an alkaline. Oxygen is fatal to one, carbonic acid to another. Tartaric or phenic acid will kill off some species more rapidly than others,* and so by repeated cultivation in conditions favourable to this or that form of microbe, and prejudicial to those with which it is found associated, you get what you want. To analyse a disease which, like septic poisoning, may result from a number of microbes, you must resort to a similar process. Some of these flourish in one animal or cultivation fluid, but die in another, and on that principle the analysis can be made. Koch, for instance, found that by injecting a drop of putrefying matter into the circulation of the common mouse, death resulted in 40 to 60 hours, and two distinct phenomena presented themselves under the microscope. The blood swarmed with vibrios of extreme minuteness, and a micrococcus arranged in peculiar chains, the smallest which Koch has ever seen, was found in the serum at the point of inoculation. On the ordinary mouse it was impossible to separate this latter microbe from the other, but by inoculating them on the field mouse Koch found that the vibrio perished and the micrococcus gave rise to a peculiar gangrene. Neither of these microbes affected the rabbit. Coze and Feltz, on the other side, selected that animal for their experiments, and they found that the inoculation of a few drops of putrefying matter into its circulation caused the blood to swarm with certain other microbes. But the curious point brought out by their experiments was that by

* According to MM. Gayon and Dupetit, in a paper read before the French Academy of Sciences almost as this is passing through the press, a microbe, which their experiments show to be the cause of the reduction of nitrates, not only tolerates strong solutions of phenic and salycilic acid, but ferments them with as little concern as yeast ferments sugar. This shows that—as with mankind—one microbe's food is another's poison, and illustrates the necessity for a more systematic investigation than has yet been attempted of the precise action of reputed disinfectants in different diseases.

repeated cultivations of these microbes in the rabbit they could enormously increase their virulence. Thus, having injected from 1 to 10 drops of blood putrefied in the air into the veins of each of 48 rabbits they found that 26 of them died. Injecting 1 to 4 drops of the blood of one of the rabbits dead in this experiment into a number of others, they found that all inoculated perished. Following the same procedure, they found that at the fifth generation one-hundredth part of a drop killed the animal in less than 20 hours. At the tenth generation they could kill a rabbit with the 20,000th part of a drop, and in subsequent generations with even smaller amounts. Toussaint claims to have proved that the organism thus cultivated out is none other than the microbe of fowl cholera, of which we shall speak hereafter.

The two forms of septic disease investigated by Pasteur are human septicemia and pyemia. In the first of these he has discovered a septic vibrio wonderfully akin in many respects to the microbe of butyric fermentation. Like that microbe, oxygen kills it, but like it, too, its germs are unimpaired even by compressed oxygen. They will germinate in carbonic gas, or even in vacuo. Like it, too, it manifests its fermentative action by the liberation of hydrogen and carbonic acid. Its habitat *par excellence* is in the serum of the abdominal cavity, and in the muscles of the abdomen. It does not pass into the blood till the last stage of the disease or after death, and in that fluid it assumes an aspect of immoderate length, frequently passing beyond the entire field of the microscope, and a translucidity so great that it often escapes observation. When, however, adds Pasteur, one has once seen it, it can easily be found "creeping, flexuous, and separating the globules of the blood as a serpent separates the grass in a thicket." This microbe does not give rise to the formation of pus, but apparently poisons its victim by the production of an alkaloid which Bergman has named sepsine. The second form of septic organism, described by Pasteur, is the pyogenic. It is evidently identical with the micrococcus, which forms the principal subject of an able and interesting memoir by Dr. Ogston, of Aberdeen. It is found in the pus of wounds and ulcers, and in that of abscesses, and the multitudes in which it exists in the latter case may be understood from the fact that Dr. Ogston, taking the average of a number of specimens, tells us they showed close on three million micrococci per cubic centimetre. Inject a drop of pus containing them and you will certainly cause abscesses to form. Destroy the organisms by boiling, or select pus devoid of them, and with proper precautions you will have no such result. Like many ferment organisms, these particular micrococci are at once aerobic and anaerobic—can multiply themselves either in presence of free oxygen or cut off from it. Their life, however, when exposed to oxygen is not a vigorous one, and when put to the test of artificial cultivation outside the living body, the slightest access of free oxygen to the fluids in which they are grown renders the results most uncertain. Having tried all manner of devices to achieve their cultivation, Dr. Ogston at last hit upon a method at once simple and ingenious. Taking a fresh egg, he drilled a minute hole through the shell at one end, and through it, by means of a slender cannula, which he passed down to the other end of the egg, he injected a drop of pus containing these micrococci. Then covering the egg with antiseptic dressing, so as to prevent the access of any organisms from without, he maintained it at a suitable temperature. On opening it, after a few weeks, he found that the micrococci had multiplied and spread themselves throughout the entire egg. A drop of its contents injected into another egg in the same manner would give rise to the same results, and a drop of the albumen thus artificially contaminated with the micrococcus, after many artificial cultivations, would give rise to its characteristic formation of pus. Of course, pus can be formed without its intervention, but when it is present the suppuration is much more abundant, and what is all important, there alone will the pus set up fresh abscesses wherever a drop of it, or the pyogenic microbe with which it abounds, may be borne by the circulation.

But you will probably think that I have long enough assumed the connection of cause and effect as existing between these microbes and the diseases with which they are found associated, and that it is time that I should say something of the methods by which it can be proved that they really stand to each other in that relation. You are aware that microbes of various species may be found in the saliva and in the secretions of the bronchi and intestines, even where there is no disease, and there can be no doubt that after death these microbes make their way into the blood and tissues of the body, when the microscope often reveals them. It is not enough, therefore, to find bacteria or vibrios, or other microbes, in the fluids of the body, to enable you to say that they have had anything to do with a disease. To prove the relation of cause and effect between a specific microbe and a specific disease a much more rigorous method of demonstration must be adopted. You must not only show that the microbe is found associated with the disease, but that it is always associated with it. You must not content yourself with showing that by the inoculation of blood or serum containing the microbe the disease can be produced, but you must show that if the microbe be destroyed or removed the fluids are rendered harmless; and to complete the case you should be able to free the microbe by artificial cultivation from all contamination with the fluids of the disease, to grow it as a simple ferment outside the body, and to show that, thus purified, when introduced once more into the animal economy, it gives rise once more to the particular disease.

The process has been most exhaustively and satisfactorily worked out in the case of the *bacillus anthracis*, the organism of the splenic fever of cattle. That microbe is exceptionally large, and it was doubtless in consequence of this characteristic that it was the first to be discovered and have its importance as a disease factor recognised; but it is a noteworthy fact in connection with my text, that Davaine was led to his discovery by a perusal of Pasteur's memoir on Butyric Fermentation. I shall dwell at some length upon the proofs that in this case the microbe is the real cause of the disease, because they illustrate the nature of the proofs which are relied on in other cases. If you inoculate a healthy sheep or calf with the blood of an animal suffering from splenic fever the operation communicates the disease. If you filter the liquid through plaster under atmospheric pressure, or through a number of sheets of filtering paper, firmly compressed together, the liquor will be found innocuous; and if you boil it long enough to kill the microbes the same thing will result. Beyond that proof you could hardly go until you could lay hold of the microbe itself, and cultivate it outside the body altogether. This Koch succeeded in doing five years ago. The cultivation fluid to which he resorted was the aqueous humour of the eye of an ox. Subsequent investigators have discovered that other media answer equally well, and thus, artificially cultivated, the whole life history of the microbe can be followed under the microscope. Like other organisms of its class, it multiplies itself by fission, and by the production of germs or spores. These processes are not hypothetical, but in the case of this bacillus, as in the case of other microbes of disease or fermentation, they have been witnessed by numbers of competent and independent observers. Multiplication by fission seems to be the mode of growth of the bacillus of splenic fever when the temperature is too high or too low for the most vigorous development of the organism, and you can at will determine its mode of reproduction by regulating the temperature at which it is cultivated. How completely, by means of a few successive cultivations, you can get rid of all trace of the fluids of the diseased animal from which the microbe is originally procured, can be understood from a statement which has been made, and which is probably sufficiently accurate for illustration, though I have not checked the calculation, that after twelve cultivations, each one in not more than ten centimetres of fluid, the primary drop of liquid is as much diluted as if you had originally added to it a volume of liquid as great as the total volume of the earth. Freed thus from every trace of original impurity, the microbe of splenic fever continues to produce the same specific results, and, thus purified, the different degrees of vitality of the organism and its germs can be verified by direct experiment. The life of this bacillus, like that of other microbes, is easily destroyed. That of its germ or spore is as defiant as theirs of ordinary destructive agencies. It preserves its vitality for years, resists drying or moisture, cares nothing for compressed oxygen or absolute alcohol, and can defy a heat considerably above the boiling point. Now, it is a fact remarkably illustrative of the intimate natural connection between microbe life within and without the animal body that it has been shown by direct experiment that if the blood of an animal which has perished of splenic fever is mingled with earth (as where one is skinned on the field where it has perished), especially if the earth be moistened with manure water, the bacillus grows and multiplies itself, and gives rise to numerous corpuscle germs, which can be recovered by cultivation many months or years afterwards, and are still capable of giving rise to the disease. In fact, Pasteur has demonstrated that it is in this manner that the disease is perpetuated in those countries where it abounds, and has shown, in the most conclusive fashion, the important part played by the earth-worm in bringing to the surface spores of the microbe from the graves in which animals that have died of splenic fever have been buried.

And now let us glance at another microbe, the study of which has gone on along with that of the microbe of splenic fever, casting upon it a light which has led to the achievement of the most marvellous practical results. I refer to the microbe of fowl cholera. This is a minute micrococcus which, as I have already said, Toussaint, who originally succeeded in cultivating it outside the body, after a very elaborate experimental investigation, declares to be identical with the microbe of the acute septic disease which we have seen artificially produced in the rabbit. If he be correct—and certainly his facts look very strong—the genesis of fowl cholera is exactly similar to what takes place in the rabbit, only in this case the micrococcus is multiplied and freed from the other microbes, which in ordinary putrefactions struggle with it for life, by a process of natural instead of artificial cultivations on a peculiarly adapted soil. The microbe, according to Toussaint, exists among a host of others in ordinary putrefactions, and swallowed by the fowl in its food, when any accidental abrasion of mucous membrane exists, it inoculates itself upon the fowl, multiplies itself within its body, is excreted with its excrements, infects the food and pebbles swallowed by its neighbours, and in the course of a few cultivations finds itself in full purity and virulence exactly as we have seen it do under a series of artificial cultivations on the rabbit. Now, the microbe of fowl cholera, unlike that of splenic fever, so far as its history is known, multiplies itself by fission only, and has never been known to produce germs or spores. There is nothing extraordinary in this, for the yeast plant does precisely the same thing—although, according to Rees, when cultivated on slices of potato or carrot, it alters its manner of multiplication, and produces spores. I have no doubt that you have all heard how, by cultivating the microbe of fowl cholera in chicken broth, under prolonged exposure to oxygen, M. Pasteur succeeded in producing a modified, or, as he calls it, an attenuated, form of organism which will impart the disease to a fowl inoculated with it in any desired degree of severity; how it can be reduced to such a degree of attenuation as to give rise to only trifling local symptoms, and how even in that condition it exercises a specific influence on the fowl on which it has been inoculated which protects it against subsequent attacks of the original disease, in the same manner as inoculation with vacine lymph protects mankind against small-pox. What I want to call your attention to is the *rationale* of the process, and the intelligent manner in which this important discovery was made. What I want to insist upon is the illustration it affords of the all-importance of the study of microbe life in fermentation, if we desire to appreciate or to control its action in disease; and in order to illustrate this point we must go back to the yeast plant.

In the course of researches undertaken for the purpose of determining the identity or non-identity of various alcoholic ferments, Pasteur discovered that if you take any of the varieties of yeast in a state of perfect purity, place them in a quantity of wort freely exposed to pure air, and allow them to exhaust the materials on which they grow, the fermentation ceases, but the yeast cells preserve their vitality for a long time. Their structure, however, becomes altered, and their energy as ferments modified. Let us suppose that after all fermentation has ceased in the vessel enclosing a yeast under the conditions I have described, a pinch of it be taken on successive days and placed in fresh wort, fermentation will be set up, but, other things being equal, its appearance after each successive sowing will be more and more tardy. The vitality of the cells so treated becomes more and more enfeebled, and it takes them longer and longer to produce that new crop on which fermentation depends. In the end they will die, but with proper treatment they may be preserved in life for a very long time, and will then be found to have undergone very remarkable alterations in their mode of growth. Years ago, when discussing the results thus obtained, Pasteur observed that the law thus discovered would probably be found to extend itself to all anaerobic ferments. What then, could be more natural than that when he ascertained that the microbe of fowl cholera multiplied itself in chicken soup nearly as freely as yeast did in wort, he should conceive the idea of treating it in the same way, and so testing whether a similar change could not be wrought in its fermentative action? He did so, and found that so long as it was afforded fresh supplies of chicken soup it multiplied itself as yeast does under similar circumstances, unchanged, and after a hundred successive cultivations proved as virulent as at the first. But if you allow it to exhaust the element on which it lives, leaving it at the same time in contact with the oxygen of the air, a change occurs precisely analogous to that which Pasteur had discovered in the case of yeast similarly treated. Its vitality as a ferment gradually diminishes, and the virulence of its action when inoculated upon fowls every week becomes less and less marked. Now, in his yeast experiments, Pasteur had found the necessity of securing his microbe in a state of absolute purity. While its life is vigorous, its superior energy enables the yeast cell to triumph over any parasites with which it may be contaminated, and to kill them or restrain their development by forcibly seizing upon the elements which they require for their growth. But when its vitality is weakened, if these parasites exist they come to the front and kill off the yeast.

Now, we know that the living body is made up of living cells, and, when a ferment is introduced into it, then a contest for existence at once arises between them and it. Had Marix in the experiment I have referred to made use of yeast enfeebled by the treatment just described, I have no doubt he would have found that he could have injected much larger doses than he did without fatal results. In the struggle for life the weakened yeast would have gone to the wall, just as the fresh yeast did when an insufficient dose was set to work against the combined forces of all the blood globules and other cells of the body. Now, this is precisely what did occur when the microbe of fowl cholera, enfeebled by prolonged starvation in oxygen, was introduced into the circulation of healthy fowls. The energy of the organic cell-life within them was sufficient to achieve over it a victory more and more easy, precisely as it was less or more enfeebled—exactly as, when infected in the ordinary way, fowls die or recover according as their natural forces are or are not sufficiently strong to overcome the dose of the microbe which has introduced itself into their system. And as it is found under circumstances of ordinary infection that the system of the animal which recovers has undergone some change which renders it unapt to contract the disease a second time, so in those which recovered from the attenuated virus it was found that a like effect had been produced.

Extending his researches to the microbe of splenic fever, Pasteur discovered that a similar course of treatment in its case produced similar results. It, too, became enfeebled or attenuated, but with much greater rapidity, and when thus attenuated its inoculation upon sheep or cattle produced precisely analogous effects. But in the case of this microbe there is an important difference, with greatly augments the facility with which we can avail ourselves of its attenuated form as a protective against the disease. As I have said, you can, by regulating the temperature of its cultivation, at will compel the microbe of splenic fever to multiply itself either by fission or spores. In order to secure the desired attenuation it is necessary that it should multiply itself by fission, and if, when attenuated to any required degree, it is allowed to produce its spores, these spores retain precisely the power as ferment factors which they have inherited from their attenuated parent cell, and months and probably years afterwards will reproduce their species with fermentative powers attenuated in the same degree. And what renders this fact the more remarkable is, that it is exactly in accord with another fact discovered by Pasteur in his investigation of the yeast plant—namely, that when submitted to a very prolonged process of starvation in contact with oxygen, its cells underwent changes very similar to those considered by Rees to mark its sporulation, and that, when then cultivated in fresh wort, the yeast resulting possessed characteristics altogether distinct from the species of microbe from which they had been proximately derived. Again, as the fermentative vigour of the attenuated yeast plant can be restored by cultivation under circumstances favourable to its growth, so the attenuated microbe of splenic fever cultivated on newly-born guinea pigs, or the attenuated microbe of fowl cholera cultivated on small birds, is rapidly reinstated in all its original destructiveness to animal life. In the course of these investigations other modes of curtailing these microbes of their powers have been discovered. Toussaint, for example found that by heating blood containing the bacillus of splenic fever up to a certain point he so enfeebled those of the organism that survived that on inoculation the blood so treated sometimes gave rise to a mild protective form of the disease, similar to that which Pasteur had secured by his system. Toussaint's method was, however, uncertain. Sometimes the microbes were all killed, when no protection was afforded; sometimes they were not sufficiently scotched, when a fatal result ensued. Again, MM. Arloing, Cornevin, and Thomas have discovered that by injecting carefully-restricted doses of the microbe of the Maladie de Chabert (a disease for a long time confounded with splenic fever), direct into the blood current of cattle, precautions being taken to prevent all contamination of the solid tissues in the process, they can produce the same protective result. On the other hand, the smallest infection of these tissues with the organism may give rise to the disease in its worst form.

How are we to account for the the difference? More than one theory has been advanced, but it is probably premature to generalise, for different disease ferments seem to act very differently under the same circumstances. Thus Chauveau found that by injecting vaccine into the circulation of the horse he produced a generalised eruption instead of the strictly local one produced by ordinary inoculation with the virus. Different disease microbes will doubtless be found to develop greater vitality in different fluids and tissues, precisely as different ferment microbes are known to thrive best in different media of cultivation. My object has been to trace the intimate connection which exists between the processes of fermentation and disease, but as yet I have said nothing of the products of fermentation in disease. That such products are formed there can be no doubt, but their production is complicated with so many other vital reactions, and their operation so masked by other vital phenomena that, so far, very little is known regarding the extent or nature of their action in disease. The microbes, multiplying themselves in the body by millions and tens of millions, as they are known to do, must materially interfere with the composition and aëration of the blood. By their size, or by the peculiarities of their growth, they may block up the capillary circulation in vital organs, and produce death by some mode altogether unconnected with the product of their fermentation. Thus the yeast injected in Marix's experiment deprived the blood of every trace of sugar, doubtless with the production of alcohol; but no alcohol was discovered in the analysis, and it probably played a very secondary part in the causation of death. That the microbes of disease may give rise to distinct ferment products outside the body is shown by the fact that the fluid in which the microbe of fowl cholera has been cultivated is impregnated with a new narcotic product, and that when freed from the microbe by filtration and injected into healthy fowls, this product gives rise to temporary symptoms of narcotism, exactly similar to those which in the natural disease constitute a very prominent feature. Certain of the microbes of putrefaction, too, in the course of their development outside the body give rise to an alkaloid —sepsine—to which I have already referred, which, as a poison, produces the same symptoms observed in certain forms of septic disease.

Did time permit I might adduce other examples of a similar kind, but it would serve little purpose; for, though the importance of the study of this phase of microbe life is now recognised, that study is as yet in its infancy. You will, however, with myself, be inclined to think that I have already trespassed upon your patience at too great length. I have by no means exhausted the subject, nor have I been able even to touch upon some very interesting points in connection with it. My object has been to trace in fuller detail than I have yet seen done the intimate connection between the processes of fermentation and disease, and to illustrate what has again and again been insisted on by Pasteur in France and Tyndall in this country, that to the right understanding of the nature and processes of the one the study of the nature and processes of the other is an indispensable requisite. I am fully aware that I have done but scanty justice to my theme, but we have considered together a number of the characteristics possessed in common by microbes of fermentation and disease. We have seen how organisms which habitually give rise to the process of fermentation may, when transferred to the interior of the living body, give rise to the phenomena of a specific, though artificial, disease. We have seen that the mixed ferment organisms of ordinary putrefaction, by a series of animal reagents, can be separated into their component species. We have seen in fowl cholera a disease, dependent on a ferment microbe, probably to be everywhere met with in ordinary putrefactions. We have seen how that microbe can be spontaneously separated from its fellows and brought to a state of wonderful purity and deadliness by cultivation within the animal body, and how when in this state transferred once more from a living to an artificial soil it again comports itself precisely like the ferment cell of ordinary yeast, multiplying itself by fission and producing a narcotic poison as that produces alcohol, becoming, like it, enfeebled by prolonged starvation in presence of oxygen, but capable, by proper treatment, of being once more revived into its full pristine activity. We have seen the bacillus of splenic fever with its natural *habitat* in a living soil, yet capable of life and multiplication in earth, and susceptible of artificial cultivation in yeast water and other organic solutions, manifesting the same enfeeblement as yeast under prolonged exposure to oxygen, and capable of restoration to its original virulence by analogous methods. We have seen how, inspired by the suggestive study of a fermentation, Lister thought out his antiseptic system, and Davaine first discovered that a microbe was the cause of a disease. We have seen by what obvious and intelligent steps Pasteur was led by the results of experiments on yeast plants to his process for attenuating the microbe of splenic fever, and his discovery of a preventative against a most formidable disease. Had time permitted I should have liked to have said something about the microbes described as associated with other diseases. I should especially have wished to refer to the proofs which Professor Villemin has adduced to show that tuberculosis is a disease of microbic origin—what different microbes have been said to have been discovered in connection with it, and how Toussaint appears at length to have discovered the right one, and has found it to increase in virulence under artificial cultivation.* I should have liked to speak of the extraordinary and important results reported by M. Talamon in connection with diphtheria, which only require confirmation by independent observers to render that formidable scourge one of the most thoroughly understood of our diseases. I should have liked to point out the manner in which the whole subject has been shown to bear upon the mysteries of hydrophobia, these again upon the nature of the poison of snake-bites and the possibility of their cure, and that again upon the existence of an unsuspected poison in human saliva. One might, in fact, advert to all these and many more topics, pregnant with promise to the future of medicine and humanity, and yet leave the subject unexhausted, with much still to be said about the part played by microbes in fermentation, putrefaction, and disease.

* This was written before the result of Koch's conclusive researches into the connection between microbes and tubercles were known in this country.

PRINTED BY GUNN AND CAMERON, FLEET STREET, DUBLIN.