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ROMANCE OF SCIENCE SERIES.

OUR SECRET FRIENDS AND FOES

PERCY FARADAY FRANKLAND

PH.D., B.SC. (LOND) F.R.S.

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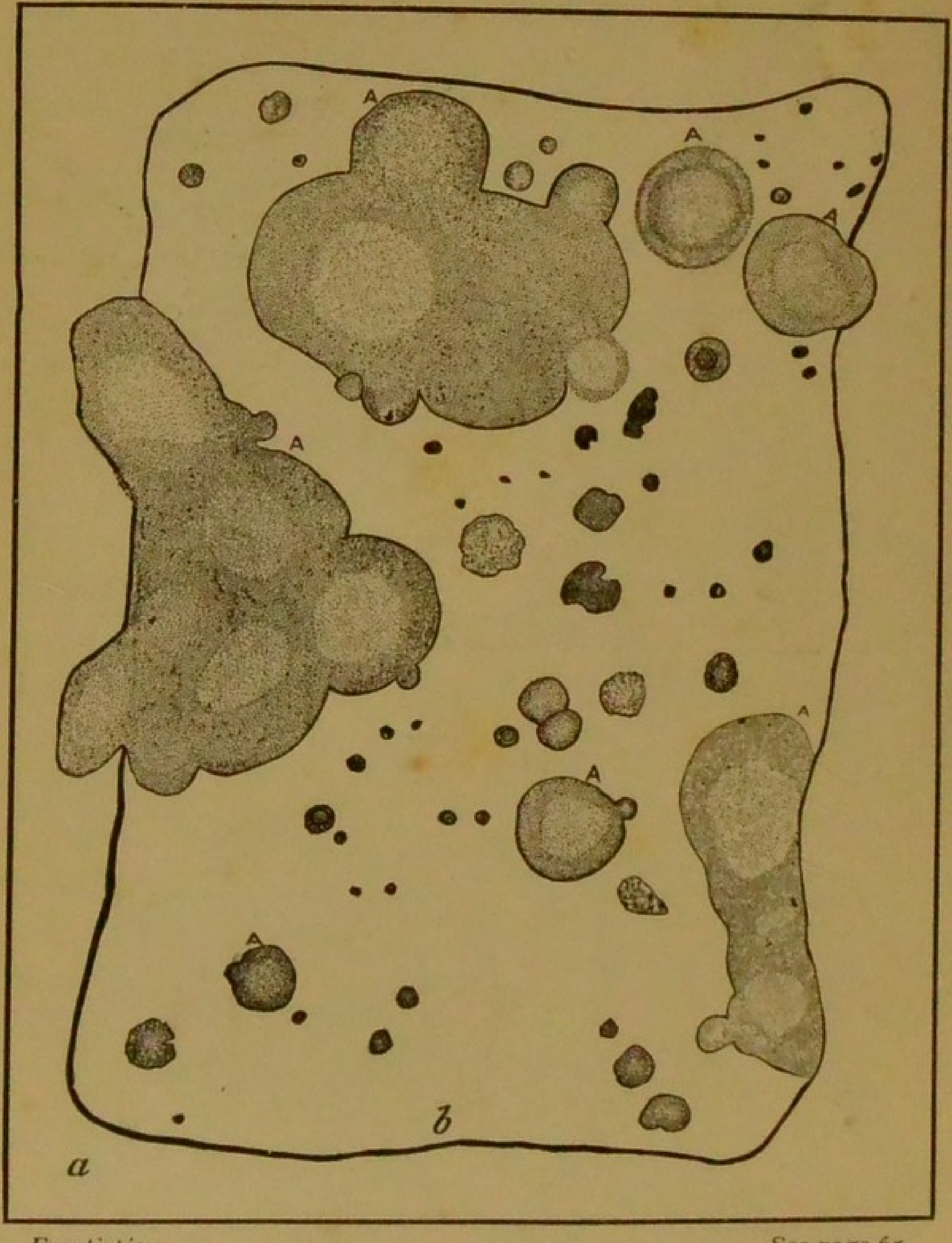
OUR SECRET FRIENDS AND FOES.



Frontispiece. See page 65.

Gelatine-plate culture, showing colonies of micro-organisms. (A) Colonies causing liquefaction of the gelatine; (*b*) represents the film of gelatine; (*a*) the glass plate upon which it is poured. (*After de Giaxa.*)

THE ROMANCE OF SCIENCE.

OUR
SECRET FRIENDS AND FOES.

EXPANDED FROM LECTURES DELIVERED
BEFORE POPULAR AUDIENCES IN LONDON, EDINBURGH,
AND ELSEWHERE.

BY
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THESE PAGES ARE OFFERED AS A SMALL TRIBUTE TO THE COMPANION WHOSE SYMPATHY AND INVALUABLE ASSISTANCE HAVE SO GREATLY ENHANCED THE ENJOYMENT OF SCIENTIFIC WORK.

PREFACE.

THE low forms of life, commonly known as germs or micro-organisms, are daily attracting more and more public attention, as a knowledge of their remarkable powers for good and evil is becoming more widely diffused, and the great strides which have been made in their careful study is undoubtedly one of the most conspicuous features in the past quarter of a century.

This little book, it is hoped, may be a means of making the general reader more intimately acquainted with this recently discovered world of life, and of affording him a glance at its hidden organization and resources.

PERCY F. FRANKLAND.

UNIVERSITY COLLEGE, DUNDEE.
November, 1892.

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OUR SECRET FRIENDS AND FOES.

I.

INTRODUCTION.

If Nature had provided us with eyes one thousand times as powerful as those with which we are at present obliged to be contented, we should form a very different idea of the living world around us. The living creatures, both plants and animals, with which we are now familiar, would fall into utter insignificance, as regards their number, by the side of the countless millions of diminutive living particles which would then come into view. These minute living particles would be seen to infest all our surroundings—floating in the air we breathe, swimming in the water we drink, and in full possession of every inch of the ground upon which we stand and walk.

But although the unaided eye cannot behold these wonders, the ingenuity of Man has enabled us, with the assistance of the microscope, to discover this new world with its overwhelming multitudes of living beings. It is to these excessively minute living creatures, or micro-organisms, as we now generally term them, and their beneficent and malignant functions in Nature, that I purpose introducing you.

In the first place, let us look into the size and shape of these tiny living forms, which the highest powers of our modern microscopes allow us to discern at least as clearly, sharply, and distinctly as we are able to observe the ordinary objects which surround us.

The minuteness of these organisms is so excessive that their dimensions baffle description in the ordinary terms of measurement. Thus, without going by any means to the smallest known forms, we find as a common length of such organisms the one-twenty-thousandth of an inch—a figure which obviously conveys no definite impression with it. Perhaps it may help you to realize it better if I tell you that no less than four hundred millions of these organisms could be spread over one square inch in a single layer. Thus we could have a population one hundred times as great as that of London settled on an area of a single square inch, without any complaint of over-crowding, and giving to each individual organism, not three acres —which Radical politicians used to tell us were necessary for the individual man—but one-four-hundred-millionth of a square inch, which is quite adequate for a citizen in the commonwealth of micro-organisms.

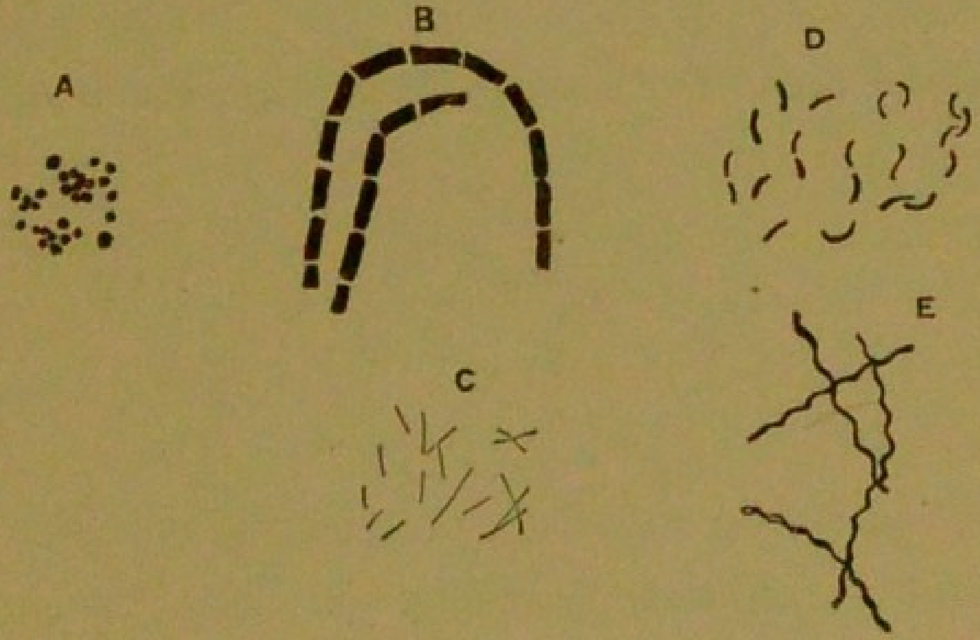

Fig. 1.—Various forms of Micro-organisms.

The shape of these "liliputians" is generally very simple. Thus, some are merely more or less spherical granules, to which we give the name of *micrococci* (see Fig. 1, A); others again, from their rod-like shape, are known as *bacilli* (see Fig. 1, B and C); whilst others, having a corkscrew, or spiral form, are known as *spirilla* (see Fig. 1, E). Such spirilla sometimes appear in a much shorter form, and then resemble the shape of a "*comma*," as seen in Fig. 1, D. All these various forms are sometimes loosely spoken of as *bacteria*. In addition to these bacterial forms there are two other classes of micro-organisms—the *Saccharomycetes* or *Yeasts* (see Fig. 2), and the *Moulds* (see Fig. 3). The yeasts, you see, are comparatively large, oval bodies, whilst the moulds consist of long threads, giving rise to the well-known hairy patches with which we are all so familiar on articles of food, such as jam, bread, and meat, which have been unduly exposed to air and moisture.

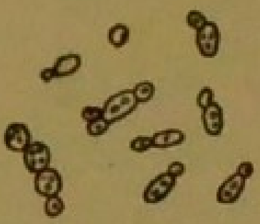

Fig. 2.—Yeast-cells.

Of these variously-shaped micro-organisms the bacilli and spirilla are motile, or capable of

Fig. 3.—*Penicillium glaucum.*

locomotion, the yeasts and moulds are stationary, whilst the micrococci, which until recently had been classed in this respect with the latter, must now, in consequence of the discovery of some motile forms, rank with the bacilli and spirilla. But by no means all of the varieties of bacilli and spirilla are motile, and it is curious how bacilli, which in almost all other respects closely resemble one another, manifest the most marked difference as regards their powers of locomotion. The movements executed by these motile organisms form one of the most fascinating and entertaining microscopic spectacles which exist. The varied motion of the countless swarms of individuals, following their sinuous paths across the field of the microscope in all directions and in the three dimensions of space, much after the fashion of a cloud of midges playing in the sunshine, produces an irresistible impression upon the observer, that each individual microbe is assisting in and conscientiously performing its part in a highly complex and thoroughly-organized Scotch reel, conducted at express speed.

Interesting and important as it is to observe the appearance of micro-organisms in a living state, yet this is by no means sufficient for the minute investigation of their form and other characteristics with a view to subsequent identification. The great advances which have been made in our knowledge of micro-organisms within recent years are largely due to the methods which have been devised for accentuating their appearance by means of brilliant colours. Striking colours are employed by both the savage and the civilized man, or rather woman, to render themselves more conspicuous, in the latter case by clothing in coloured garments, whilst by the savage the process of brilliantly colouring the body itself is adopted. Now this is also the process employed by bacteriologists to render micro-organisms conspicuous amongst their surroundings. Their bodies are dyed, and the dyeing is carried out on the same principles as the dyeing of a skein of silk or a hank of wool.

These textile fibres, silk and wool, as is well known to dyers, are most readily coloured by what are called the *Basic Colouring Matters*, to which group belong the brilliant *aniline dyes* like magenta, methyl violet, malachite green, etc.

The great advantage of these dyes consists in the rapidity and intensity with which they colour the materials for which they have an affinity.

It must not be supposed that all materials have an affinity for or can be dyed by these Basic Aniline Colours, and this difference in the behaviour of some substances enables us to distinguish micro-organisms, which readily take up and retain the dye, from their surroundings.

In most cases, however, the staining of micro-organisms is not such a simple matter as this, for the bodies with which they are surrounded have also generally a strong affinity for these basic colours, which results in their becoming also dyed with the colour employed. There is, however, a peculiarity about these micro-organisms which enables us to strongly dye them whilst leaving the surrounding matter colourless or only faintly tinged. The microbes in fact appear to be invested with an envelope which offers great obstacles to the penetration of the colouring matter to their interior. This envelope of the micro-organisms, however, serves to protect them from influences which readily affect bodies not so surrounded, and it is this circumstance which is taken advantage of in various ways in the dyeing or staining of bacteria.

Thus one of the simplest and most commonly-practised expedients consists in exposing the micro-organisms, and the materials with which they are mixed, to such a temperature that whilst the other materials are so scorched by the heat that they are no longer capable of being dyed, the micro-organisms, wrapped in their protecting blanket, so to speak, suffer practically no injury. On now applying the dye to the scorched specimen, the micro-organisms become strongly and permanently coloured, whilst the surrounding matters remain colourless.

It might possibly be thought a very difficult operation to apply heat so delicately adjusted as to injure these other materials without affecting the micro-organisms, but as a matter of fact with a little experience it can be done with great readiness and facility.

The examination of stained micro-organisms, dyed as I have described, sometimes reveals the presence within the micro-organisms of small round or oval bodies, which have not taken up the colouring matter. These bodies are the so-called *spores* (see Fig. 4), which, in consequence of their great power of resisting destruction, are of such tremendous importance in the propagation of some micro-organisms, and we shall have to refer to them in greater detail later on. Although sufficiently conspicuous by the fact of their not sharing in the gay colours which have been bestowed on the micro-organisms themselves, bacteriological artists are nevertheless fond of painting them also, which can be done by a little modification of the process I have described.

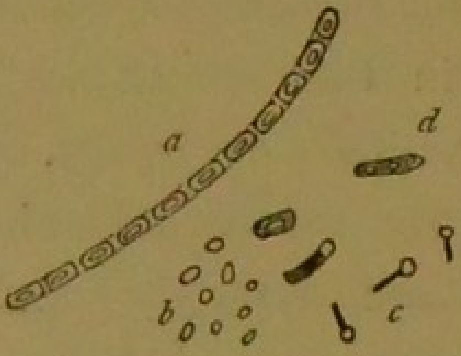

Fig. 4.—Spores: (*a*) chain of bacilli, each containing an oval spore; (*b*) free spores; (*c*) bacilli, each with a spore at its extremity; (*d*) swollen bacilli containing spores.

To this end the preparation containing spores is dyed, not with an aqueous solution of one of the basic colouring matters, but with a solution of the colouring matter in aniline-water, which gives the dye a greater power of penetration. In this manner even the spores become dyed, and *à fortiori* the fully-developed micro-organisms also. The colouring matter, having penetrated into the interior of the spores, is there much more firmly fixed than elsewhere in the preparation, so that if we now submit the latter to a decolourizing process by immersion in dilute nitric acid (1 : 3), the colour will be removed from the spores last.

Having then removed the colour by means of dilute acid from everything excepting the spores we proceed to re-dye the fully-developed micro-organisms by means of another colour in the ordinary way. In this manner a beautiful and most effective microscopic preparation is obtained in which the spores are tinted with one colour and the fully-developed organisms with another.

It is a highly remarkable phenomenon that some micro-organisms offer the same difficulties in the way of being stained as do the spores. This is notably the case with one of the most important of all the micro-organisms with which we are acquainted, viz. the *tubercle bacillus*. The great value of detecting this bacillus for clinical purposes has led to a number of methods being devised for rendering them apparent through staining. These methods are all based on essentially the same principle as those which I have described for the staining of spores.

The refinements of microscopic technique in connection with micro-organisms have, however, gone even beyond what I have described, for none of the methods of staining already mentioned are successful in displaying certain highly-important structures possessed by some forms, viz. the organs of locomotion by means of which some at least of the motile microbes are able, as we have seen, to propel themselves about in liquid media, often with fabulous rapidity. These organs of locomotion, unlike either the bodies or the spores of micro-organisms, have no affinity for these aniline colours, which we have seen are so useful in exhibiting the latter. In the practice of dyeing textile fibres we are also acquainted with fibres which exhibit the same indifference to these colours; thus cotton and linen cannot be directly dyed with the Basic Aniline Colours, and in order to dye them with these colours we have in the first instance to prepare these fibres by impregnating them with what are known as *mordants.* Now this is essentially the principle on which it has been found possible to demonstrate in the most beautiful way the existence of these organs of locomotion, or *Flagella* as they are called (see Fig. 5).

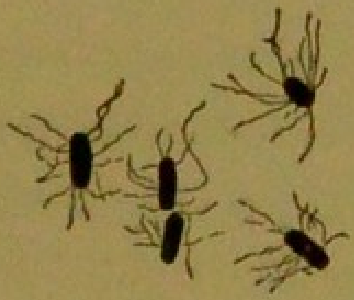

Fig. 5.—Bacilli of Typhoid Fever, showing the Flagella or organs of locomotion. (*After Migula.*)

In the accompanying figure a bacillus is seen, from the walls of which most peculiar wavy threads start out in all directions. These are the Flagella, and in some varieties extend, as in this case, in considerable numbers from all parts of the body. In some instances they give rise to a bushy appearance either at one or both ends of the microbe, whilst they are also found single or in pairs (rarely in threes) at either pole. The above drawing is taken from a preparation of the bacillus of typhoid fever made and photographed by Dr. Migula of Carlsruhe.

It will thus be seen that we are now provided with numerous appliances for carefully studying the form of micro-organisms; indeed, with the best modern microscopes and by careful staining, their outlines are so sharply distinguishable that the most beautiful photographic representations of these minute forms of life are now obtainable.

The process of reproduction amongst the micro-organisms is generally a very simple one, and takes place under favourable conditions with enormous rapidity. The spherical micrococci merely become constricted by a waist, which, becoming more and more fashionable, results in the formation of two distinct bodies from one (see Fig. 6). The multiplication in the case of the bacilli is perfectly similar, the division taking place transversely to their length. In many cases, however, the bacilli are capable of another and highly important mode

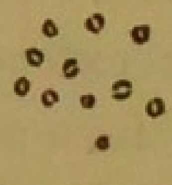

Fig. 6.—Micrococci, showing mode of multiplication.

of multiplication. In the interior of the bacillus there appears a round or oval body, already referred to, having a very bright and shining lustre (see Fig. 4). This bead-like body is known as a *spore*, and plays a most important part in the propagation of many kinds of bacilli; for just as the seed is much more enduring than the plant from which it is derived, so these spores are capable of resisting many hardships which would be immediately fatal to the parent bacilli from which they have sprung. Thus these spores will endure the severest privations both of hunger and thirst, they are unaffected by cold far greater than that of an Arctic winter, and will sometimes survive a few minutes' exposure to boiling water—*in fact, such spores are the hardiest forms of living matter which science has yet revealed.*

Having become superficially acquainted with the general appearances of these minute forms of life, we will now endeavour to gain a more intimate knowledge of their habits, and for this purpose we will next consider their distribution.

II.

MICRO-ORGANISMS IN AIR.

THAT the air we breathe is more or less laden with living organisms is a fact which is far from acceptable to most of us, and yet it would require but little persuasion to convince the majority of mankind that air *without* organisms would be undesirable indeed; for without one micro-organism at least, which is very widely distributed in the air, we should have to forego the numerous and complex uses of alcohol in its various forms.

But there are other micro-organisms in the air besides yeast, and it is the firm conviction that many infectious diseases are propagated by means of air-carried microbes, that renders the investigation of the subject of aërial micro-organisms peculiarly interesting and attractive. Passing over a number of isolated observations by Leeuwenhoek, Ehrenberg, and Gaultier de Claubry, the systematic examination of the aërial microbia commences with those marvellous discoveries with which the name of Pasteur is so inseparably connected, and with which the latter half of the nineteenth century will for ever be associated. These now classical researches of Pasteur's on the presence of micro-organisms in the atmosphere, were undertaken in connection with the fierce controversy which raged some thirty years ago, on the "*Spontaneous Generation of Life.*"

As most of you are doubtless aware, it was contended by the teachers of this doctrine, that the presence of the smallest particle of air was sufficient to determine the generation of low forms of life in certain highly-putrescible substances, such as milk, blood, infusions of meat, and the like. The opponents of this doctrine, marshalled by M. Pasteur, contended, on the other hand, that it was not the air, but certain living germs in the air, which, gaining access to these putrescible materials, gave rise to those growths which make their appearance in them.

"Were this the case," replied his antagonists, the preachers of spontaneous generation, "these aërial germs would give rise to a fog as opaque and impenetrable as steel." But Pasteur was not to be shaken in his conviction by dogmatic and baseless assertions of this kind, and he therefore undertook to prove his case by experiments so clinching and unanswerable, that they could leave no shadow of doubt in any unbiassed mind.

The methods by which Pasteur not only revealed the presence of micro-organisms in the atmosphere, but, at the same time, roughly mapped out their distribution, are so striking in their beautiful simplicity, that I must run the risk of being charged with telling again an "oft-told tale," and refer to them in detail.

The apparatus employed by Pasteur for this purpose consisted of a number of small flasks. Into these flasks, which were about one-quarter of a pint in capacity, was introduced a small quantity of what is known as a cultivating medium, *i.e.* a material in which these lower forms of life are capable of flourishing and multiplying abundantly. The cultivating medium employed in this case was clear broth, which forms an excellent nourishing material for the greater number of known micro-organisms. The necks of these flasks were then drawn out to a fine aperture (see Fig. 7), and the contents heated to boiling for some time, with the double object of *sterilizing*, or destroying, any living matter in the culture-medium on the one hand, and, on the other,

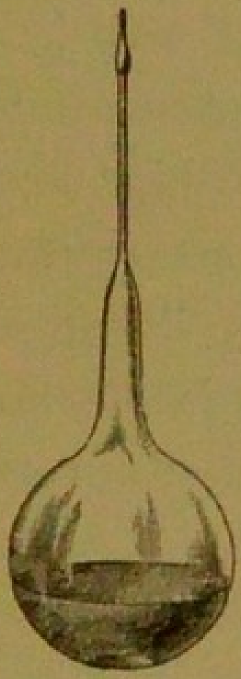

Fig. 7.—Pasteur Flask.

of driving out the air from the flask. The open extremity of the flask was then closed by heating it in the flame of a blow-pipe, whilst steam was still issuing from the mouth.

Pasteur found that the cultivating material in flasks prepared in this way remained sterile, or, as we should say in homely language, "sweet," for an indefinite period of time.

With a collection of flasks of this kind, Pasteur explored the atmosphere of a number of different places; for by breaking off the drawn-out extremities of the flasks in any given place, the vacuum inside the flask is immediately filled with the air in question, bringing with it all that it holds in suspension. The broken point is then re-sealed at once before the blow-pipe, and, on preserving the flask at a suitable temperature for a few weeks, the presence of any living organism which has gained access along with the air will manifest itself by its growth in the broth rendering the liquid turbid.

Now Pasteur exposed—

Twenty flasks in the open country of Arbois.

Twenty more on the lower heights of the Jura Mountains.

Twenty more at the Montanvert, close to the Mer de Glace, at a height of upwards of 6000 feet.

These three series of samples were deposited by Pasteur in the bureau of the Academy of Sciences, in the month of November, 1860. The result, which was awaited with the utmost interest by the numerous *savants* who had taken part in the previous discussions, was as follows:—

Of the twenty flasks opened in Arbois, eight developed living organisms.

Of the twenty flasks opened on the Jura, five became affected.

Whilst of the twenty flasks opened on the Montanvert, only one broke down.

Thus Pasteur succeeded in not only proving the truth of his previous assertions, but demonstrated for the first time the greater relative purity of air at high than at low levels.

Striking as these results are, they can only boast of having roughly mapped out the difference, from a microbial point of view, of the air in these places; they make no claim to determining the actual number of organisms in a cubic foot or any other given volume of the specified air. Since these first experiments of Pasteur's, numerous endeavours have been made to raise the examination of air for micro-organisms from a *qualitative* to a *quantitative* one. Amongst the earlier workers in this direction we must mention Miquel and Freudenreich, who devised a method based upon the use of liquid culture media, by means of which they carried out a very large number of experiments, and their results may lay claim to a fair amount of accuracy *inter se.*

Since, however, the masterly researches of Robert Koch have brought so prominently before the world the beautiful methods of cultivating these micro-organisms on a solid instead of on a liquid medium, the problem of approaching the study of the aërial microbia has undergone a fundamental alteration.

The first adaptation of these new methods to the exploration of the air was made by Koch, who exposed dishes containing sterile solid cultivating material, the organisms falling upon which subsequently gave rise by their growth and multiplication to little patches or centres visible to the naked eye. These centres or "colonies," as they have been expressively designated, exhibit often very characteristic appearances, producing very various colours, and growing often in fantastic shapes ; their number can thus be readily counted, and the members belonging to each colony can be separately studied.

The number found on dishes thus exposed gives no clue to the number of micro-organisms present in a given volume of air, but can only indicate the quantity falling on a given surface in a given length of time. One particularly interesting point was established by the use of this method, for it was found that the colonies consist almost invariably of one family; that they are, in other words, pure cultivations, thus clearly showing that the different varieties of organisms present in the air are separated from each other, and do not occur adhering together.

In conducting experiments on the distribution of organisms in air, I made large use of this method, and employed small circular glass dishes, rather less than one inch in height, and about three inches in diameter, provided with a glass cover fitting loosely, and overlapping like the lid of a pill-box (see Fig. 8). These were filled to a depth of about one-third of an inch with nutrient jelly. This "gelatine-peptone," as it is called, is composed of the finest of French gelatine, to which is added meat extract, common salt, some peptone, and distilled water. It is deprived of all organic life by being heated for half an hour on three successive days in a steam sterilizer (Fig. 9). In this manner it is surrounded by an atmosphere of steam, and becomes evenly heated throughout the entire volume without the liquid actually boiling, a point of great importance in connection with the preparation of such culture materials. The idea of allowing an interval of time to elapse between the several heatings up of the liquid is due to Dr. Tyndall, who, realizing the difficulties presented by the remarkable powers of endurance possessed by the spore (to which we have already referred), thus allowed time for it to develop into a bacillus, in which form it readily succumbs to the action of heat. It was presumed that in two days all the spores originally present in the liquid would have grown out into bacilli, and that the third day's sterilization would finally dispose of these more

Fig. 8.—Culture-dish.

readily destructible forms. This has been amply verified by experience, and the science of bacteriology owes an enormous debt of gratitude to Dr.

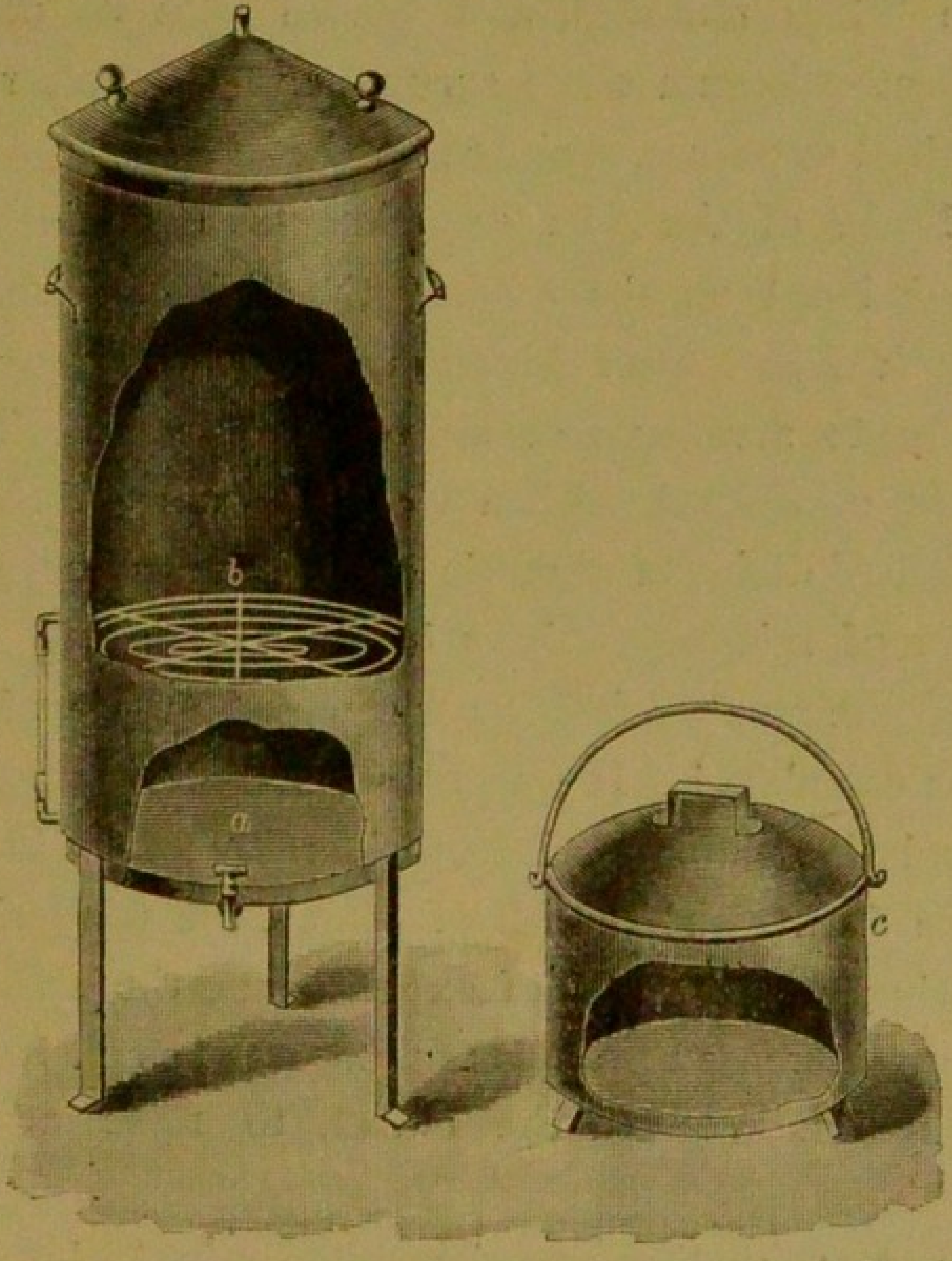

Fig. 9.—Steam Sterilizer: (*a*) water-chamber in which the steam is generated; (*b*) wire shelf on which the objects for sterilization are placed; (*c*) convenient vessel for holding the objects to be sterilized; it is provided with a wire bottom only, so as to admit of the circulation of steam through it.

Tyndall for the introduction of this process, now known as "intermittent sterilization."

In using dishes of gelatine-peptone thus prepared, the lid is removed, and placed with its mouth downwards on a clean surface, and then, after the desired exposure, replaced on the dish.

Some of the first experiments I made were carried out on the roof of the Science Schools, South Kensington Museum, being a distance of sixty to seventy feet above the ground. They were conducted with a view to determining the effect of different climatic conditions on the numbers of micro-organisms in the air, and in each case the number falling per square foot per minute was calculated. It was found that during a high wind the numbers rise considerably; thus in one case during the month of March, the wind rising during a succession of experiments, the numbers found increased from 851 falling on one square foot in one minute at the outset, to 1,302 at the finish.

But on another occasion, when rain had fallen, and the ground and surroundings were thoroughly wet, the numbers found amounted to from 60 to 66 per square foot per minute. Again, during a thick white fog there were present only from 26 to 32. I shall have, however, later on, in connection with the estimation of the aërial microbia in a given *volume* of air, to enter into a more detailed account of the variations occurring over a whole year, and will, therefore, pass on to some investigations made in the interior of buildings by the "dish" method.

We should naturally suppose that when many people are gathered together and much movement is going on, we should find the number of micro-organisms an increasing one. Now, that this is actually the case, is shown by the following experiments made at Burlington House, in the rooms of the Royal Society, during a Conversazione very largely attended. At the commencement of the reception, there were 240 microbes falling on a square foot in one minute, but as the rooms became more crowded, the numbers rose to 318, whilst the following morning, when there were only three persons in the room, and everything was covered with dust, the number was reduced to 109.

This point is further strikingly shown by experiments made at the Natural History Museum, South Kensington. The dishes were exposed in the centre of the large entrance hall one Monday morning in May, and again in the afternoon of the same day, when there were considerably more visitors than in the morning. The numbers found were respectively 30 and 293. But these figures sink into utter insignificance when compared with those obtained on a Whit Monday. This being a general holiday there was a very large number of visitors in the building, consequently the amount of disturbance was proportionately great, and the enormous number of 1,755 micro-organisms was found to be falling on a square foot in the course of one minute. The air was also similarly tested during a children's dance. At the beginning, when but a few children had arrived, only 44 were found, but later on, when dancing was going on energetically, this number increased to 400.

Again, in the Richmond Ward (containing eight beds) of the Hospital for Consumption at Brompton, there were found 18 in the morning, but later on in the afternoon, when more people were moving about, there were as many as 66.

But even these experiments, striking as they appear, are thrown into the shade by comparison with the following figures, obtained under circumstances which would seem to promise as rich a microbial harvest as could possibly be obtained. I was travelling in a third-class railway carriage from Norwich to London, and soon after leaving Norwich I tested the air; there were at the time four persons in the carriage; one window was closed, the other open, and the experiment was made near the open window. Under these circumstances 395 organisms were found to be falling on the square foot in one minute. On reaching Cambridge, the carriage was taken possession of by a number of men returning from Newmarket races. The carriage remained quite full (ten persons) to London. About half-way between Cambridge and London I made a second experiment, one window being shut, and the other was only open four inches at the top; the air was tested near the closed window, with the result that no less than 3,120 organisms were found to be falling on the square foot in one minute. But this number even was exceeded in an experiment made in a barn in which flail thrashing was going on. The atmosphere was visibly laden with dust, and on testing it with a gelatine dish, I found that upwards of 8,000 organisms were falling on the square foot during one minute.

We must now turn our attention to the estimation of the number of micro-organisms contained in a definite volume of air. I have referred briefly to the liquid culture methods of Miquel and Freudenreich in this direction, but must now pass on to the adaptation of solid media to this purpose.

To rightly appreciate the principle of the "Hesse tube" method, which was the first application of solid media for the volumetric estimation of the aërial microbia, I must go back to some very striking experiments made some years before by Dr. Tyndall, which although more especially directed towards tracing the connection between some of the optical properties of air, and the presence of living organisms, yet revealed the very important fact that in calm air they require a comparatively short time to subside. As is well known, when a powerful beam of light is passed through the air of an ordinary room, the path of the beam is rendered visible by the illumination of

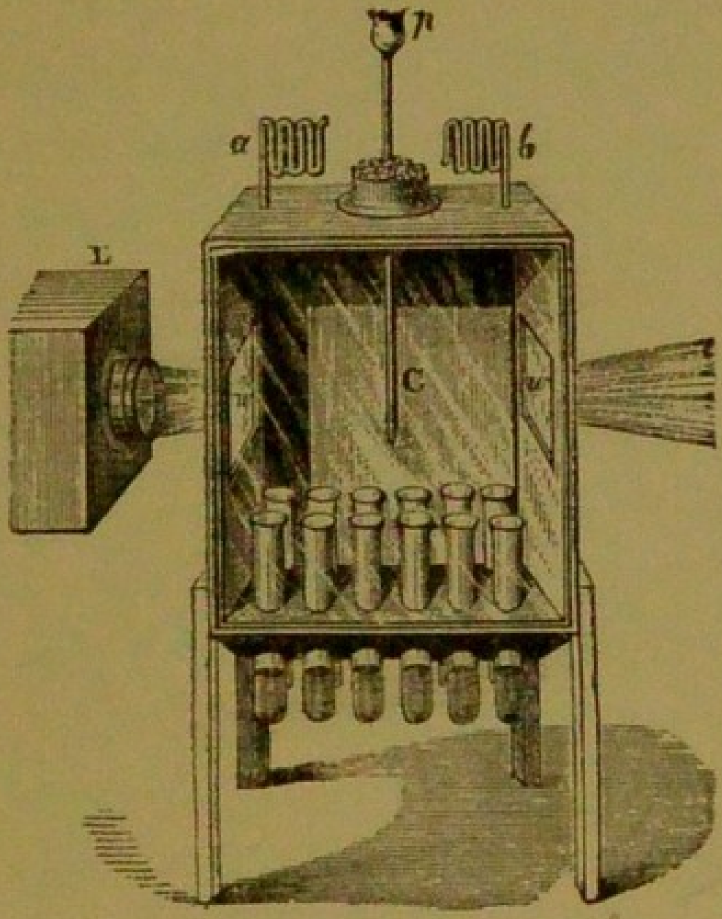

Fig. 10.—Tyndall's Germ-free Chamber. Twelve tubes are shown, containing putrescible materials, like broth, hay-infusion, &c. These liquids have been introduced through the funnel *p*, which can be directed so as to deliver into any one of these tubes. *a* and *b* represent serpentiform tubes, by means of which alone the air inside the chamber is in communication with the outside air. L represents a lantern throwing a powerful beam of light, which traverses the chamber through the glass windows, *w w*. The path of this beam within the chamber is invisible, owing to the absence of suspended particles.

a vast multitude of floating particles. Now, Tyndall found that if the air in an enclosed chamber be allowed to remain at rest for some time, and a beam of light is then passed through the chamber, its path is no longer visible, the air within the chamber being free from suspended particles capable of reflecting and dispersing the light of the beam. He further showed that this "moteless air," as he called it, was incapable of causing alteration in boiled broth and other cultivating media, or, in short, that the moteless air was sterile or free from micro-organisms.

Now it is this remarkable gravitating property possessed by micro-organisms, thus so conclusively demonstrated, which Hesse has taken advantage of, and which is, in fact, the principle upon which his whole process depends. He makes use of a glass tube about 2 feet 6 inches in length, and 1½ to 2 inches in diameter, coated on its internal surface with the nutritive gelatine medium. One end of this tube is fitted with a perforated aperture 0·5 inch in diameter, the other extremity being provided with a tightly-fitting india-rubber cork, through which passes a short glass tube, plugged with cotton wool. The perforated cap is covered with a second non-perforated one, which is tightly wired on so as to be water-tight. The tube is

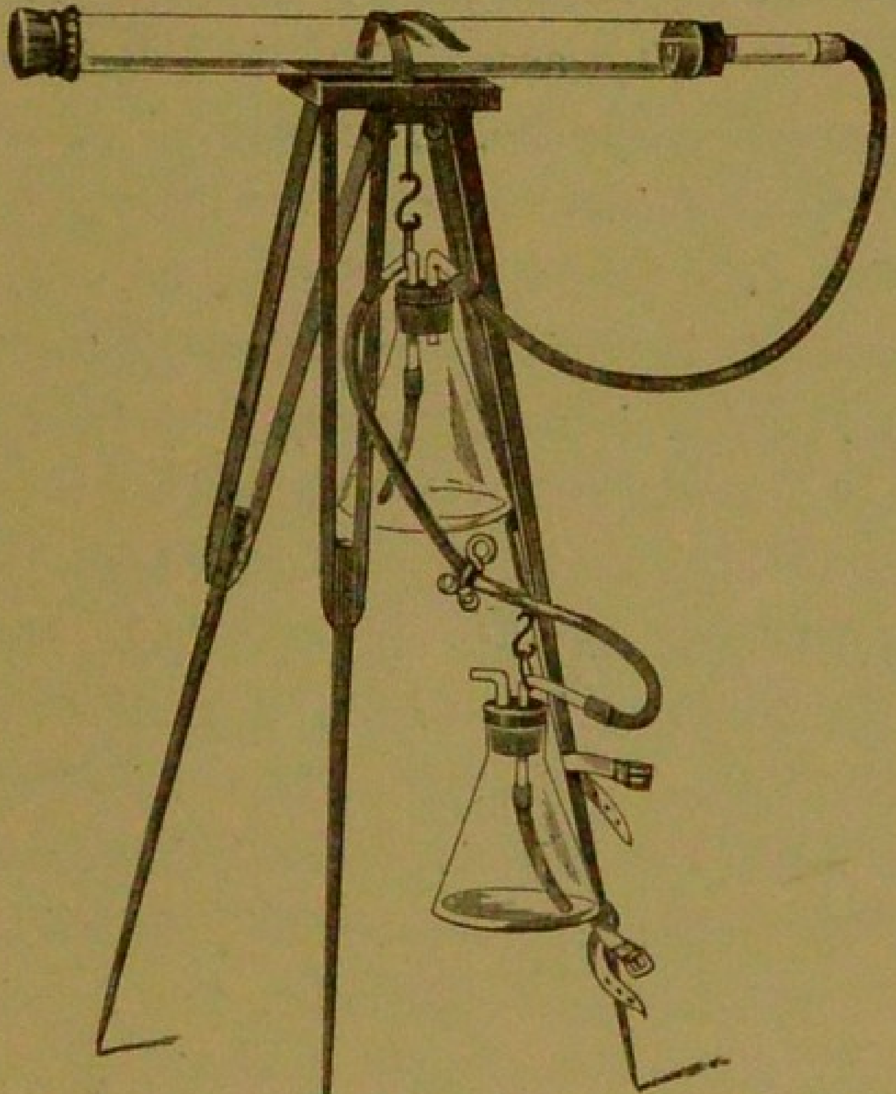

Fig. 11.—Hesse's Apparatus for estimating the number of Micro-organisms in a given volume of air.

strapped to a small horizontal table supported by an ordinary portable camera stand, the end of the small tube passing through the cork is connected by flexible tubing with an aspirator, which, so as to render it as portable as possible, consists of two bottles or strong flasks, each of rather more than one quart capacity, arranged as in the figure to form a reversible syphon. A measured volume of water is poured into one of these flasks, and by syphoning this volume into the second, an equal volume of air is made to pass through the tube. The rate of flow is regulated by a screw clamp, and by alternately connecting the end of the tube with the two syphon flasks, any desired volume of air may be drawn through the apparatus. Hesse's experiments show that the rate of aspiration should not exceed one litre in two or three minutes, and that when this precaution is observed, the organisms present in the air are almost wholly deposited in the first two-thirds of the tube, the remaining third being either wholly free or practically so. In the experiments which I conducted with Hesse's method, I fully confirmed the fact of the remarkably complete deposition of the organisms in the front part of the tube, as well as of their being almost uniformly found on the bottom. In commencing the experiment the outer unperforated india-rubber cap is removed and carefully folded up, so that its inner surface is not exposed to the air, and as soon as the experiment is over it is carefully replaced and wired on. In the course of a few days the number of organisms deposited are readily distinguishable by the colonies visible to the naked eye, or with the aid of a magnifying-glass, to which they give rise. From the number found in a given volume of air the number in any standard volume can be calculated.

I must now pass on to some of the results obtained by this method, and for this purpose I will draw your attention to the following diagram, which embodies observations made on the roof of the Science Schools, South Kensington Museum, over an entire year. You will observe that although the number of micro-organisms in the air frequently undergoes great changes from one day to another, yet the general tendency is for the number to follow the temperature, and that it is during the hottest months of the year—July and August—that the largest number of micro-organisms is present in the air.

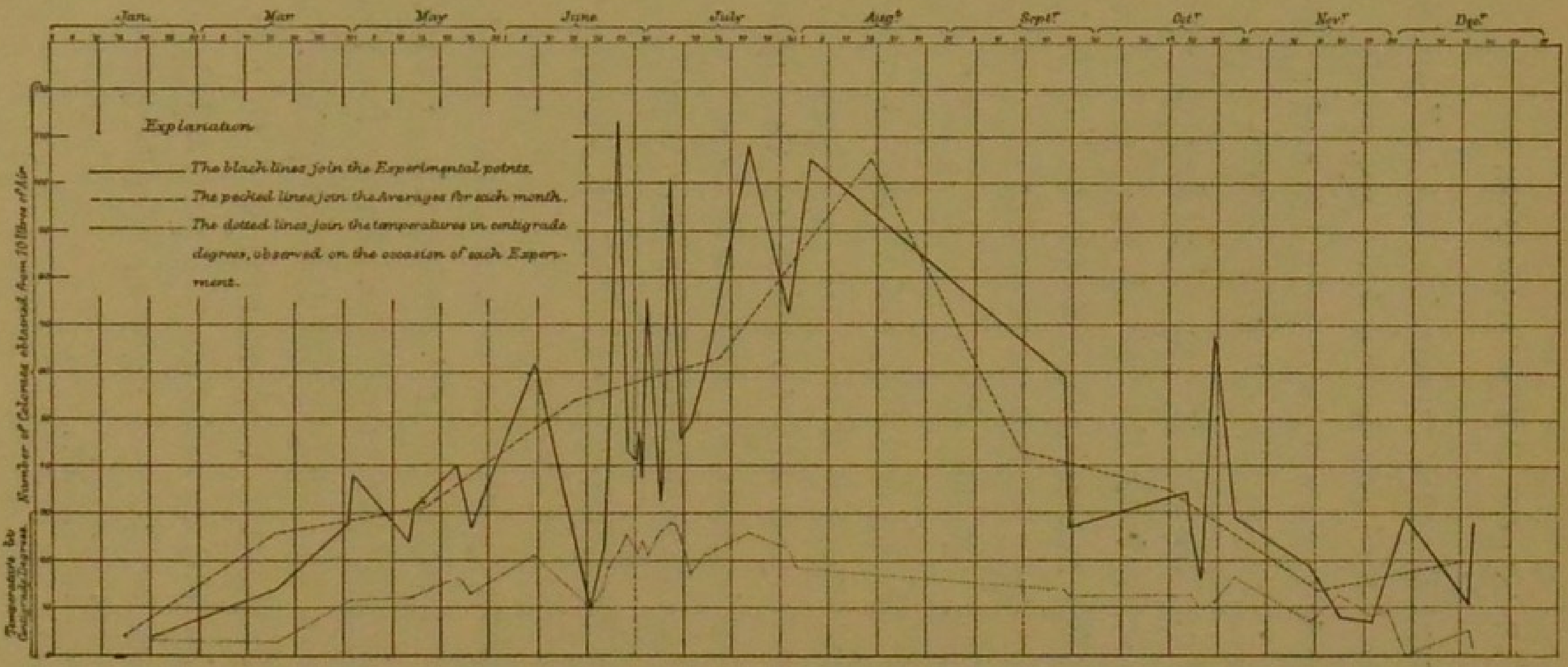

Fig. 12.—Curve showing the monthly variation in the number of aërial Micro-organisms during the year 1886. (*Percy Frankland.*)

I employed this "tube-method" also for the determination of the number of microbes present in air at different altitudes. You will remember that Pasteur already was able roughly to indicate the greater microbial purity of the air high up than on the plain, and the following results fully confirm these early experiments. The spire of Norwich Cathedral is rather more than 300 feet high, but I was only able to get up to a height of 300 feet; even this, however, was sufficient to demonstrate the purity of the air, for in 2 gallons only 7 micro-organisms were found; rather lower down on the cathedral tower, at a height of 180 feet from the ground, 9 were deposited; whilst on the gravel space in front of the cathedral, 18 were found. These experiments were made on one and the same afternoon, so that the results are strictly comparable *inter se.* The dish results were even more striking, for whereas 49 organisms were falling per square foot per minute at the elevation of the spire, at the tower there were 107, and on the ground as many as 354. Experiments made in London at St. Paul's showed the same marked difference. The air at the Golden Gallery contained 11 in 2 gallons; at the Stone Gallery, 34; and in St. Paul's Churchyard, 70; the "dish" experiments yielding respectively 115, 125, and 188. Even the humble elevation of Primrose Hill, Regent's Park, was found to be of importance in this respect, for at the top the tubes yielded 9 in 2 gallons, whilst at the foot there were 24 in the same volume. The dishes confirming these results gave the figures of 12 and 57 respectively.

In striking contrast to the number of micro-organisms found in the various places referred to above, is the number found in the air at sea. Some examinations of sea-air have been made by Dr. Fischer, a surgeon in the German navy, and as he has used substantially the same methods as those described, his results may be fairly regarded as comparative. Dr. Fischer's experiments may be thus summarized:—

I. Fourteen experiments, with an average of 22 gallons of air, contained no organisms.

II. Five experiments, with an average of 16 gallons of air, contained 1 organism.

III. Two experiments, with an average of 22 gallons of air, contained 2 organisms.

IV. Three experiments, with an average of 28 gallons of air, contained 3 organisms.

V. Six experiments, with an average of 12 gallons of air, contained 4·13 organisms.

They may, however, be more instructively classified by taking into consideration the distance from the nearest land at the time of the observations, thus:—

Maximum distance from land in sea-miles, 90; 1 organism to 5 gallons of air.

Minimum distance from land in sea-miles, 120; 1 organism to 18 gallons.

Moreover, out of 12 experiments, made at a minimum distance of 120 sea-miles from land, in 11 the air was absolutely germ-free, and in the remaining one only a single organism was found; whilst out of 12 experiments made at a maximum distance of 90 sea-miles from land, in 7 cases organisms were demonstrable, and in only 5 cases not demonstrable. In 3 of these latter 5 cases, however, although the land actually nearest the ship was less than 90 miles, the nearest land in the direction from which the wind was coming was in two cases upwards of 500 miles, and in one case

200 miles. In the other two the land was, in one instance, 60 miles, and in another only 8 miles distant, but in this case the land in question was only a small, slightly-cultivated, and thinly-populated island. This clearly demonstrates, what we should have been led from *à priori* considerations to anticipate, that the principal factor is the distance from land in the direction from which the wind is proceeding. It would further appear that the maximum distance to which, under ordinary circumstances, micro-organisms can be transported across the sea lies between 70 and 120 sea-miles, and that beyond this distance they are almost invariably absent. Of particular interest in these experiments is the very distinct manner in which they show that the micro-organisms which are present in sea-water are not communicated to the air, excepting in the closest proximity to the surface, even when the ocean is much disturbed.

An extended experience with Hesse's method convinced me that it was not without various defects, which besides militating against its convenience also affected its accuracy, and it was with the intention of overcoming some of the difficulties which were presented by Hesse's process, that I devised the so-called "Flask method," and employed it in carrying out all my later experiments. The air under examination is aspirated through tubes about 5 inches in length, and ¼ inch internal diameter. The front end (A) of the tube is open, the other extremity (B) being slightly narrower. At a distance of 1 inch from the extremity (A) the tube is constricted so as to form a support for the first plug *a*, which is placed just in front of the constriction. At a distance of 2½ inches from the plug *a* the tube is again constricted to form a support for the second plug *b*, whilst resting against the constricted extremity (B) there is a third plug *c* of cotton-wool, which is designed to prevent the introduction of micro-organisms at this extremity of the tube.

Plug *a* is invariably made more pervious than *b*, so that any organisms which may be carried by the current of air

Fig. 13.—Glass tube containing sterile plugs through which the air is aspirated. (*Percy Frankland.*) (*a*, *b*) glass-wool plugs for collection of aerial micro-organisms; (*c*) cotton-wool plug.

through *a* shall find a greater resistance in *b*, and thus, if *b* is found to be altogether free from organisms, it clearly shows that they must have all been arrested by *a*. In order to procure this relationship between the plugs *a* and *b*, the former is constructed of a small quantity either of ordinary glass-wool, or of glass-wool which has been previously coated with cane-sugar, by soaking it in a saturated solution of the latter and then drying. The plug *b*, on the other hand, is constructed of fine sugar or glass-powder (passed through a sieve of forty meshes to the linear inch), supported in front and behind by a layer of glass-wool, either plain, or coated with sugar as above. Each of these plugs is about the size of a pea. Such tubes, ready packed with their plugs, are placed in a tin box, and the lid closed and placed in an oven and exposed to a temperature of 130° C. for three hours; they can then be kept for any length of time, and easily transported, without fear of contamination, to the place where the experiment is to be performed. When required, the sterilized tube, prepared as above, is carefully taken from the tin box, and is only handled by its extremity (B). The latter is now attached by means of a piece of stout india-rubber tubing to a piece of lead tubing about ten feet in length, which is clamped at this end (B) to a retort-stand or other convenient vertical support, whilst the other extremity of the lead tube is attached to a T-piece, by which it is connected, on the one hand, with an exhausting syringe, and on the other with a mercury pressure gauge, both syringe and gauge being mounted on a rigid support placed upon the ground.

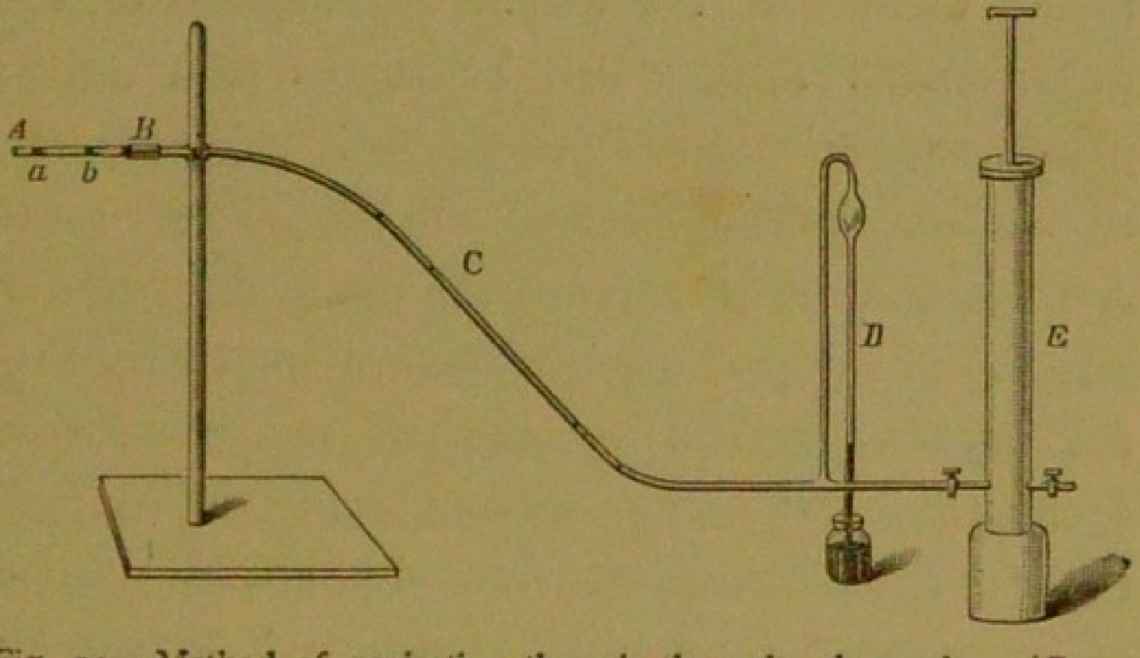

Fig. 14.—Method of aspirating the air through glass tube. (*Percy Frankland.*) (A, B) glass tube; (*a*, *b*) glass-wool plugs in ditto; (C) metal tube connecting glass tube with air-pump; (D) pressure gauge; (E) air-pump.

By bending the lead tubing, the experimental tube (A B) is brought into a horizontal position, and in out-of-door experiments the open extremity (A) is turned away at an angle from the wind. A control-tube is attached by means of wire to the vertical support, so as to rest in a precisely similar position to the experimental tube. The long piece of lead tubing enables the operator to aspirate the air by means of the hand-pump without his movements disturbing the air in the vicinity of the experimental tube, whilst by means of a mirror placed obliquely on the ground, he is able to watch the rise and fall of the mercury in the pressure-tube. After each upward stroke of the pump, the operator waits until the pressure is equalized before making the down-stroke, and by observing this precaution each stroke of the pump corresponds to the passage through the experimental tube of a definite volume of air, which is determined once and for all by means of a gas-meter. Thus, the number of strokes measures the volume of air aspirated, whilst the number of strokes performed per minute indicates the degree of perviousness which the plugs possess. The volume of air aspirated is varied, of course, according to the number of microbes supposed to be present, but with ordinary London air 60 strokes of the pump, or about four gallons, were found to be convenient. When the desired volume of air has been aspirated through the tube or tubes, the

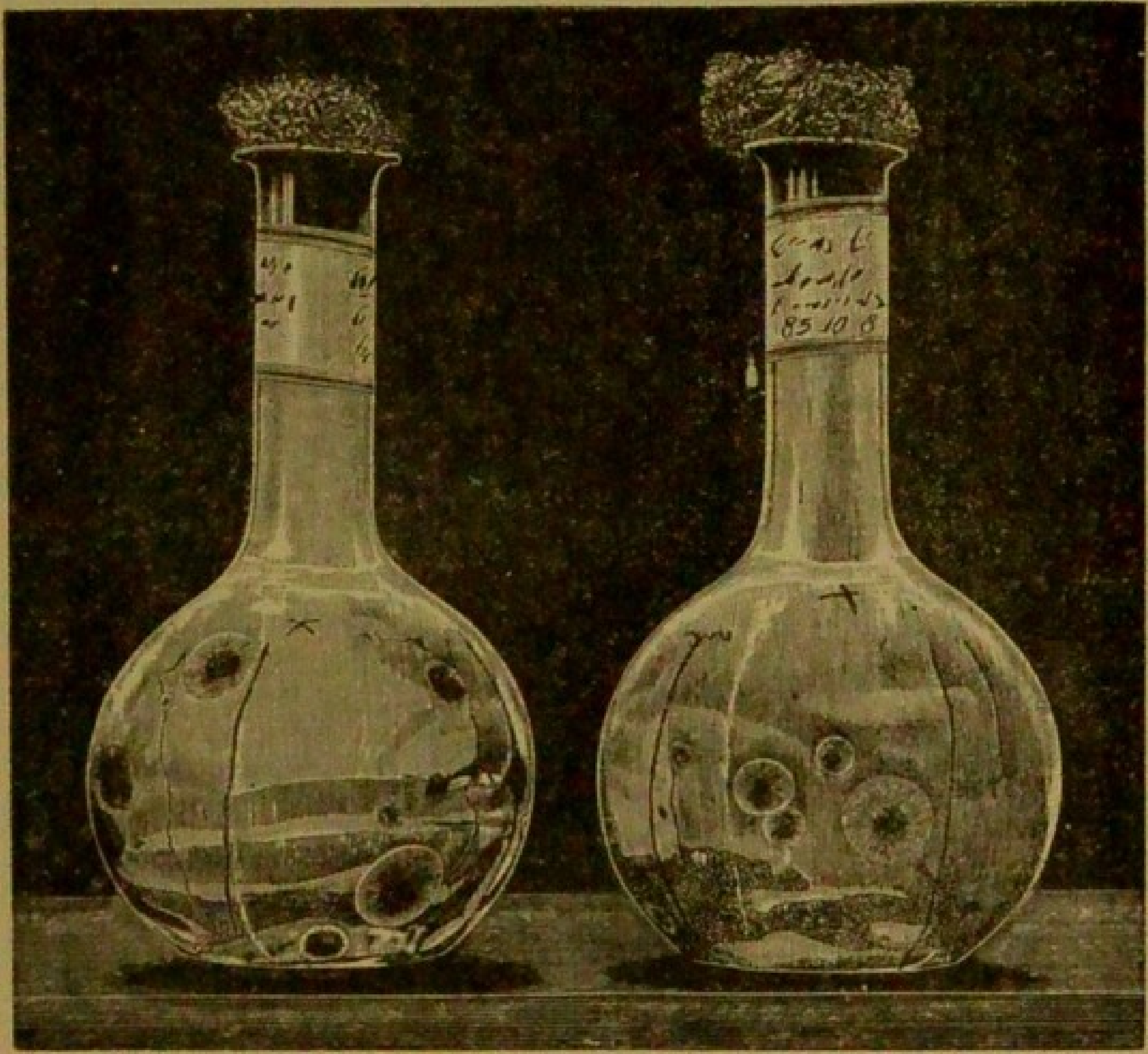

Fig. 15.—Flasks containing a film of gelatine, upon which the aërial micro-organisms are growing. (*Percy Frankland.*)

latter are at once replaced in the sterile tin box. Flasks, similar to those in the diagram, containing sterile gelatine-peptone which has been rendered fluid by being slightly heated, are employed for the subsequent development of the microbes caught on the plugs, in the following manner. The tube for examination is carefully removed from the box, and a scratch made with a file intermediate between the plugs *a* and *b*, and the tube broken across. The second half, containing plugs *b* and *c*, is carefully laid aside on a sterile support, whilst the first half is held between the thumb and first finger at the constriction, and the broken edge passed two or three times through a Bunsen flame, care being taken that the heat does not reach the plug. The cotton-wool stopper of one of the flasks is now withdrawn, and the extremity (A) of the tube is held vertically over the open flask, whilst the plug is carefully pushed down by means of a strong piece of sterilized copper wire, introduced from behind through the broken end of the tube. The plug *c* at the extremity (B) is then transferred to a second, and the plug of the control-tube to a third flask.

The gelatine in each flask is then rotated, which, whilst rapidly and completely disintegrating the plug, does not cause the gelatine to froth. This takes place in a few minutes, and the flask is then held almost horizontally under a stream of water, and by uniformly rotating it an almost perfectly even film of gelatine is spread over its inner surface. After being kept at a suitable temperature the colonies begin to make their appearance. They are easily counted by dividing the flask with ink into segments, and holding them up against the light. The diagram illustrates the appearance of these flasks after the colonies have made their appearance.

By this "Flask method" the microbial contents of a comparatively large volume of air can be collected in a very short space of time, for whilst the aspiration of two gallons of air through Hesse's apparatus takes about three-quarters of an hour, by this method about ten gallons can be drawn through the tube in the same time, whilst by numerous comparative experiments it has been found to yield results under certain conditions greatly superior in accuracy to those obtained by the "tube-process."

Before leaving the subject of air it will be interesting to gain some idea of the appearances which some of these microbes give rise to when examined under the microscope, and when cultivated in different media. One of these micro-organisms, a bacillus (see Fig. 16 *d*) not more than one-twenty-thousandth of an inch in length, can, by combining the efforts of countless myriads of individuals, produce a most magnificent display of colour when grown on various cultivating media. The intensely blood-red appearance which it presents, has in the past doubtless been the cause of phenomena which at the time were regarded as being elaborated by a miraculous agency, for this *bacillus prodigiosus*, as it is called, finds a suitable soil for its growth and multiplication on bread and other farinaceous articles of food; thus it has not unfrequently taken up its abode on the sacred wafer, and by there producing this marvellous colour has given rise to the appearance known as the "bleeding host," causing superstitious terror and alarm amongst the ignorant. Fig. 16 *a* represents its appearance as a single colony growing on the surface of the gelatine, magnified about one hundred times under the microscope; *b* is a smaller colony growing in the depth of the gelatine. Its edge is very irregular, and its contents are finely granular. After it has been growing for a short time on the gelatine it causes the latter to liquefy. This property of liquefying the gelatine is due to the peptonizing action of the growth on this material, and is possessed by some

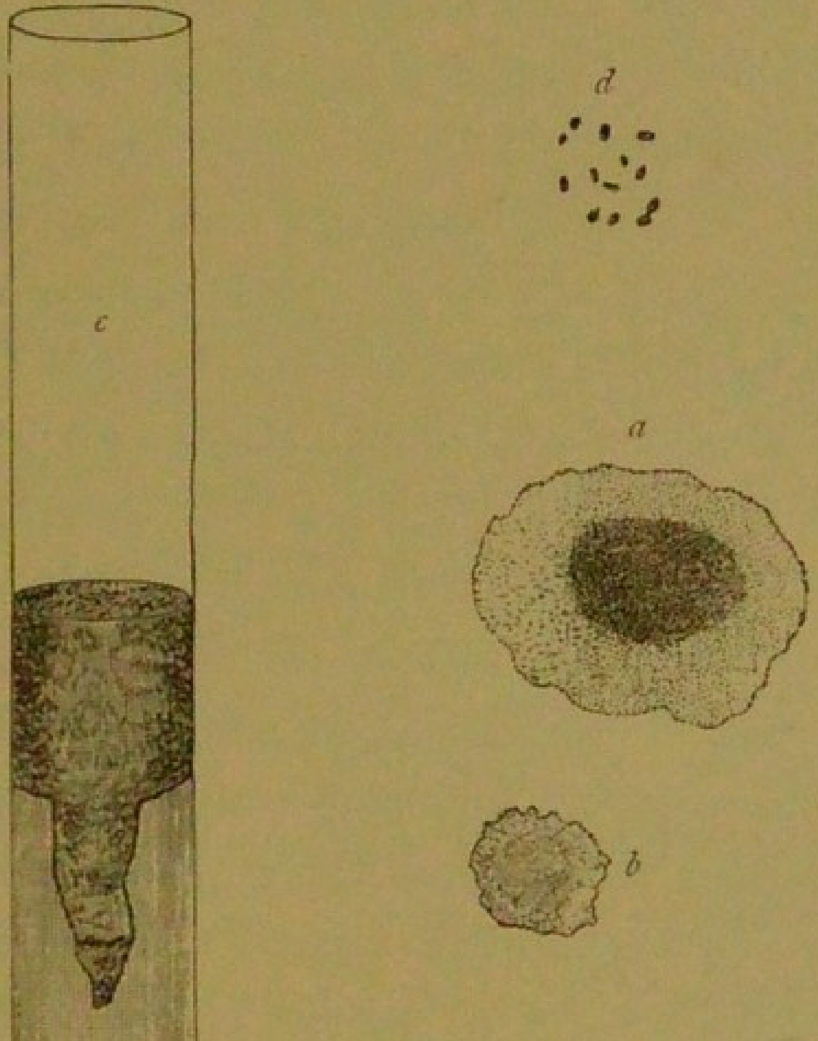

Fig. 16.—*Bacillus prodigiosus:* (*a*) colony growing on the surface of the gelatine; (*b*) colony growing in the depth of the gelatine; (*c*) a gelatine-tube cultivation; (*d*) individual bacilli magnified about 1000 times. (*After Grace C. Frankland.*)

varieties of micro-organisms, but not by others. In Fig. *c* you see what effect it produces when growing in a glass tube partially filled with gelatine. It grows very rapidly indeed, and liquefies the gelatine in the form of a conical sack, which soon extends across the tube at the top, and, gradually passing downwards, involves its whole contents.

But perhaps one of the most common of the aërial microbes is the *Bacillus subtilis*, or the well-known Hay-bacillus, so called because it can be readily procured from hay-infusions. It differs entirely from the *b. prodigiosus*, as will be seen by referring to the next figure. The colonies are some of the most beautiful and characteristic which are known. *a* represents a colony also magnified about one hundred times, growing in the depth of the gelatine; its contour is irregular, with short, spinose extensions in parts of the circumference, and the interior of each centre has a wavy structure, as if composed of coiled threads. But still more curious are the colonies of the type *b*, composed of parallel bands of fine threads, arranged in a most fantastic pattern; in the Fig. *c* the two types of colonies are united, whip-like extensions are seen ramifying out

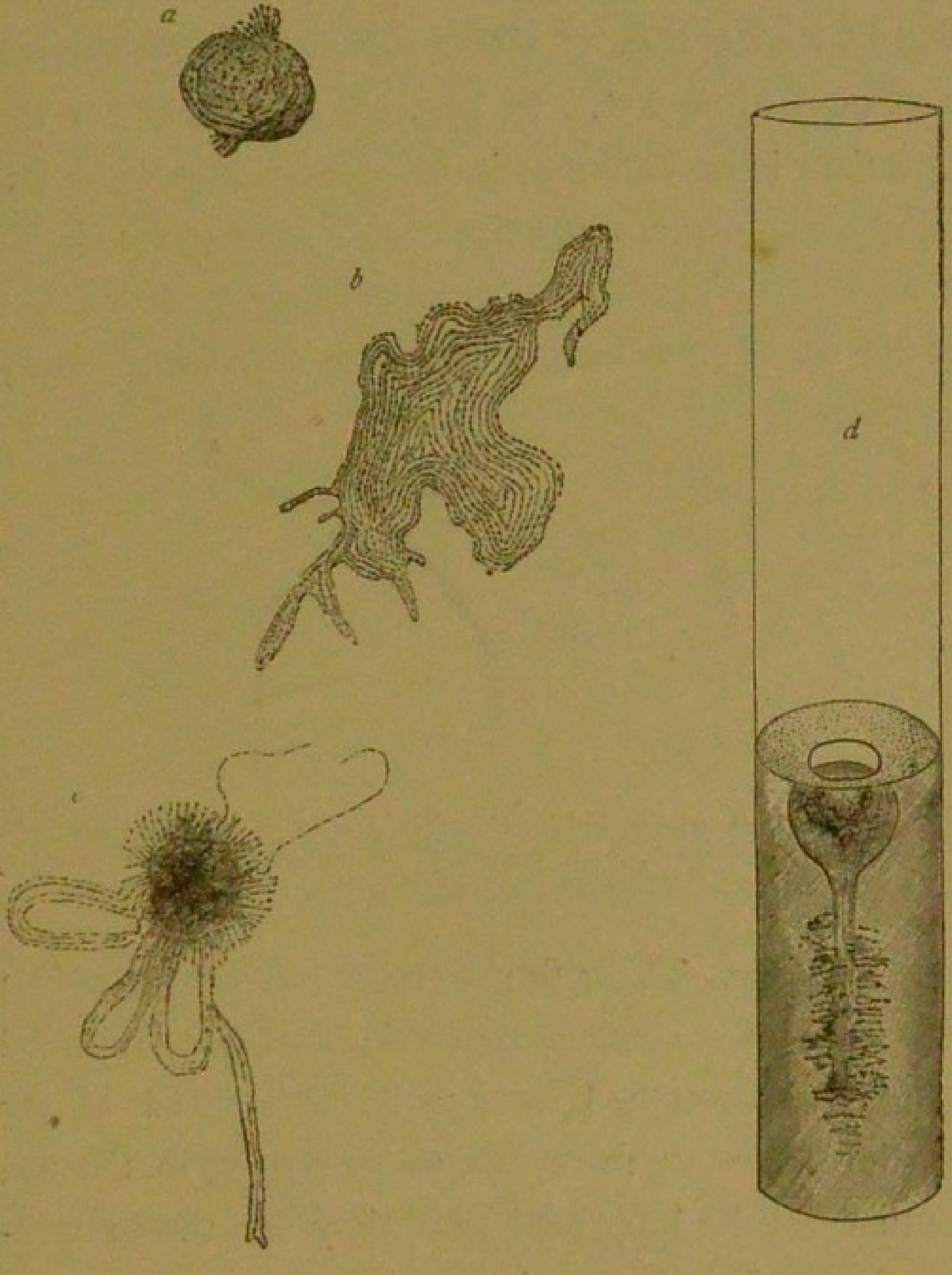

Fig. 17.—*Bacillus subtilis:* (*a*) small colony in the depth of the gelatine; (*b*) surface extension; (*c*) ditto; (*d*) growth in gelatine-tube. (*After Grace C. Frankland.*)

into the space around. Although it likewise causes liquefaction of the gelatine, yet on comparing Fig. 16 *c* with Fig. 17 *d* the difference in its growth is at once apparent. In Fig. 18, at *a*, *b*, and *c* the organism is represented in its various stages of development; some of the thicker swollen forms visible at *b* and *c* indicating the process of spore-formation, whilst at *a* the bacilli are seen occurring singly and in chains. These figures represent the organism under the microscope magnified about one thousand times. Although I have only mentioned so far bacillar forms of microbes as occurring in the air, it must not be imagined that they are the prevailing forms found; on the contrary the micrococci moulds and yeasts often far outnumber them.

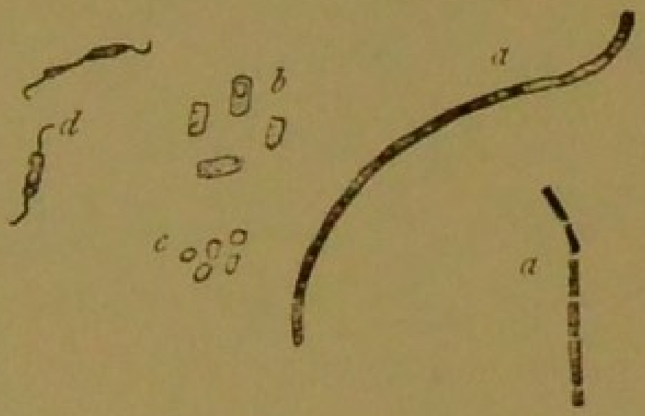

Fig. 18.—Various stages in the development of *b. subtilis:* (*a*) individual bacilli, also joined together; (*b*) swollen forms containing spore; (*c*) detached spores; (*d*) single bacilli, showing flagella.

Micrococci are found hanging together in very characteristic fashions, sometimes in chains varying in length, when they are called *streptococci*, sometimes in pairs, as *diplococci* (see Fig. 19 *b*), and sometimes in packets of four and upwards, when they are known as *Sarcina*. In Fig. 19 *a* are seen such packets of cocci. The original organism was obtained from air collected on the roof of the Science Schools, South Kensington Museum.

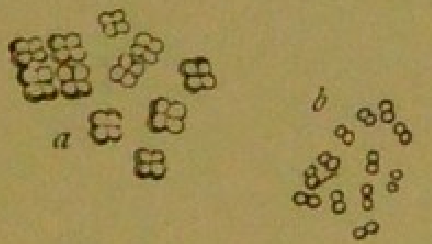

Fig. 19.—(*a*) *Sarcina*; (*b*) *Diplococcus*.

I have had a number of varieties of micro-organisms collected from the air, under more or less close observation, for upwards of six years, and it is exceedingly remarkable how constant and distinct, for one and the same organism, are the growths to which they give rise. Some of the colonies, especially, have often very beautiful and characteristic appearances, in many cases exhibiting magnificent patches of colour—deep orange, chrome yellow, brown, various shades of red, green, black, etc. Often under a low magnifying power they are seen to spread over the surface of the gelatine, producing tangled networks of threads; sometimes they resemble the petals of a flower, sometimes the roots of a tree, or its branches, constantly surprising the observer by the novelty and beauty of their modes of growth.

The investigations on aërial microbia, so far as they have as yet been carried, are of service in indicating how we may escape from all micro-organisms, whether harmful or harmless; and, secondly, how we may avoid the conveyance of micro-organisms into the atmosphere from places where pathogenic forms are known or likely to be present. This acquaintance with the distribution of micro-organisms in general, and the power of controlling their dissemination which it confers, has led to the vast improvements in the construction and arrangement of hospital wards and of sick rooms generally, and has directed attention to the importance of avoiding all circumstances tending to disturb and distribute dust. It is, moreover, this knowledge of the distribution of micro-organisms in our surroundings which has formed one of the foundations for the antiseptic treatment of wounds—that great step in surgery with which the name of Sir Joseph Lister is indelibly associated.

III.

MICRO-ORGANISMS IN WATER.

THE micro-organisms present in water have long been studied by direct observation with the microscope, but such observations can only be made in the case of foul waters in which bacterial life is very abundant, and even in such cases the information gained by the microscope alone has but little value. It is again to Dr. Robert Koch that we must acknowledge our indebtedness, who by introducing the beautiful process of "plate-cultivation," has rendered the greatest possible service in the investigation of a number of questions bearing on the micro-organisms in water. This method of plate-cultivation consists in taking some of the liquid or other substance under examination and mixing it with melted gelatine-peptone in a test-tube, the mixture being then poured out on a horizontal plate of glass and allowed to congeal, the plate being afterwards preserved in a damp chamber at a suitable temperature. In some cases, instead of plates, it is more convenient to use circular glass dishes, similar to those employed in air-investigation. In the course of a few days the colonies make their appearance, and can be counted and further studied as required. (*See* Frontispiece.) By submitting a definite volume of water to this examination we can obtain a picture of the microbial contents of different waters, and are thus enabled to attack many interesting problems, amongst others to judge of the action upon such micro-organisms of various purifying processes, artificial and natural. One of the first things we find is that different kinds of water possess these aquatic forms in very different numbers. Thus the river Thames, in its raw condition, before undergoing any treatment at the hands of the water companies, has, on the average taken throughout an entire year, as many as 20,000 micro-organisms in one cubic centimetre (or about 20 drops) of water.

Now it used to be formerly supposed that the processes of purification as carried on at the works of the water companies, which consist in storage in large reservoirs with subsequent filtration through sand, were of little if any use at all. But on examining this same water after it has been in the hands of the water companies, I found that it contained, on delivery from the mains, in the same number of drops, on an average for the same period of time only 400. We have here then the efficiency of sand filtration put practically to the test, and the assurance that it forms a very material protective measure against our infection by water-carried microbes.

In deep-well water derived from the chalk, the numbers found are usually very small indeed. Thus, in the water obtained directly from the wells sunk into the chalk, I have found an average of 18. If this result, which, it must be remembered, has been obtained from water which has undergone no *artificial* filtration, but is the raw and untreated water as we find it, be contrasted with that mentioned above—viz., 20,000, which is the average for river waters, such as those of the Thames and Lee—it is at once apparent what an enormous difference there is in the microbial condition of these waters.

Thus, excellent as are the results obtained by the artificial filtration of water through sand, yet they are even surpassed by the exhaustive filtration through vast thicknesses of porous strata such as is offered by chalk.[1] Pasteur has shown that it is not unfrequently the case for waters obtained from deep wells and deep-seated springs to be entirely destitute of organic life, or, in other words, quite sterile. But of a number of waters of this kind, I have only met with such as closely approached to this ideal state of things.

It might be anticipated that the samples of river-waters richest in micro-organisms would be those collected in the summer months, the higher temperature being more favourable to their growth and multiplication. The reverse is, however, the case; for the samples collected in the winter months were found to be, almost invariably, far richer in this respect. It must, however, be remembered that in summer the rivers are principally fed by

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[1] In this connection I may refer the reader to the tables (taken from my reports to the Local Government Board) at the end of the book, showing the number of micro-organisms which I have found in the waters of the Thames and Lee before and after filtration, as well as in the deep-well water of the Kent Company.

spring-water, and receive comparatively little surface-drainage, whilst in winter the rivers are generally more or less in flood, and are swollen by large volumes of surface-water, which is naturally rich in micro-organisms.

Another very interesting point which has been brought into prominence in the course of such water investigations, is the power of multiplication possessed by micro-organisms either naturally present in water or artificially introduced for the purposes of observation and experiment. The following figures will give an idea of the activity of some microbes in this direction :—A sample of the Kent Company's deep-well water on the day of collection contained 7 in 20 drops ; after standing one day at 20° C. there were 21, and after being kept three days at the same temperature the numbers had risen to 495,000.

Again, on one occasion some ordinary distilled water, which is water as chemically pure as it can be rendered, was inoculated with a few drops of diluted urine, and the numbers determined, with the result that they rose from 1073 in one cubic centimetre to 48,100 after standing forty-eight hours. A large number of experiments have also been made on the vitality of particular species of micro-organisms, more especially those associated with disease and called "pathogenic forms," in waters of different chemical composition, and I will briefly refer to a few of the results obtained.

Perhaps the most typical and well-defined of the micro-organisms connected with particular diseases is the *bacillus anthracis*, the exciting cause of malignant pustule or wool-sorters' disease in man, and splenic fever in cattle. I will not now enter into a detailed description of this microbe, as we shall have later on to become more intimate with it under the class of malignant micro-organisms. This bacillus on being introduced into ordinary drinking-water survives only a few hours, whilst its spores, as we should anticipate, are not in any way affected by such immersion, and retain their vitality, even in distilled water, for almost an indefinite length of time. In polluted water, such as sewage, on the other hand, not only do the bacilli not succumb, but they undergo extensive multiplication. Similarly Koch's "comma bacillus," so well known to us all as associated with Asiatic cholera, was found to flourish in sewage, being still present in large numbers after eleven months' residence in this medium. In deep-well and filtered Thames water, on the other hand, although the "comma bacilli" were still demonstrable after nine days, they were only present in small numbers. Much less vitality is exhibited by the micrococcus of erysipelas when introduced into waters of various kinds, for even in sewage this organism could not be detected after the fifth day, whilst it could not survive a few hours' immersion in purer waters.

Quite recently an Italian has made some interesting investigations on the behaviour in different waters of the bacillus which produces that terrible disease called lockjaw or tetanus. He has found that whilst in distilled water it does not lose its virulent properties, if it is put into ordinary water in which other bacteria are present, its activity is weakened, recovering itself, however, as soon as the vitality of the other bacteria is diminished.

Sea-water, by reason of its chemical composition, causes a deterioration of the bacillus, but it regains its full degree of virulence as soon as it is restored to suitable conditions of temperature and nourishment. This tetanus bacillus is then, as regards its immersion in water, a very hardy form of micro-organism, but we shall have later to consider its behaviour under other conditions, for much important light has been thrown upon the methods of successfully combating its pathogenic properties.

From these experiments it appears, therefore, that whilst as a rule ordinary drinking-water does not form a suitable medium for the extensive growth and multiplication of these particular pathogenic forms, yet, that in the condition of spores they are extremely permanent in any kind of water, however pure.

Before closing the subject of water-bacteria, it will be interesting to learn what is known about their presence in sea-water. Some very exhaustive and interesting experiments have been quite recently published by an American gentleman who made the Gulf of Naples the centre of his observations. Mr. Russell finds that the number present in ordinary sea-water is distinctly smaller than in the same volume of fresh water, and that their presence is not restricted to any particular zone, but appears to be evenly distributed throughout the whole depth which was examined; but the contents of the mud at the bottom of the sea was invariably far richer in bacteria than the water in the immediate neighbourhood of the deposit; and that this increase was not occasioned by accretions from the land, but was due entirely to the growth and multiplication of the bacteria in the mud itself. For example, at a depth of about 400 feet, he found ten micro-organisms in a cubic centimetre of water; whereas in the mud in direct contact with this water as many as 200,000 were discovered in the same volume.

In connection with the suitability of such mud deposits for harbouring micro-organisms, it has been lately found that the mud at the bottom of the Lake of Geneva was very rich in microbes, and amongst them were found numerous pathogenic forms, whilst even more recently an investigator states that he has discovered the bacillus of tetanus in the mud at the bottom of the Dead Sea.

It is curious that whereas in air micrococci prevail, in water bacillar forms predominate. I am tempted in conclusion to show you one water-organism, on account of the exquisite beauty and

Fig. 20.—*Bacillus arborescens:* (*a*) first appearance of colony growing on gelatine-plate; (*b*) more advanced stage; (*c*) individual bacilli taken from a broth-cultivation; (*d*) ditto taken from a gelatine cultivation. (*After Grace C. Frankland.*)

individuality of its growth on gelatine-plates. I found it originally in London water, and more recently in Loch Katrine and other upland waters, such as that of the river Dee, which is supplied to Aberdeen. In consequence of its characteristic appearance, I called it *bacillus arborescens*. When grown on gelatine-plates, already after twenty-four hours the colonies look, when seen with a low power under the microscope, like a branch with fine twigs growing from it at both ends; later on the "twigs" become much thicker and bushier, especially towards both ends, and finally they branch out in every direction, leaving only a small cloudy nucleus, whilst the gelatine in its immediate neighbourhood becomes soft, and assumes the appearance as seen in the figure. The bacillus itself gives rise to long and wavy threads when grown in broth-tubes (see Fig. 20 *c*). In the next figure are seen colonies in various stages of development formed by another water bacillus, viz. *b. violaceus*, so called on account of the violet-coloured pigment which it produces.

The interest attaching to the presence of micro-organisms in water originated principally in the proof, which has been furnished by medical men, that some zymotic diseases are communicated through drinking-water. In the case of two diseases, at any rate, the evidence may be regarded as conclusive on the main point, and the communicability of Asiatic cholera and typhoid fever forms one of the cardinal principles of modern sanitary science, which year by year is becoming more widely recognized and generally accepted. The germ theory of zymotic disease, which has become more and more firmly established during

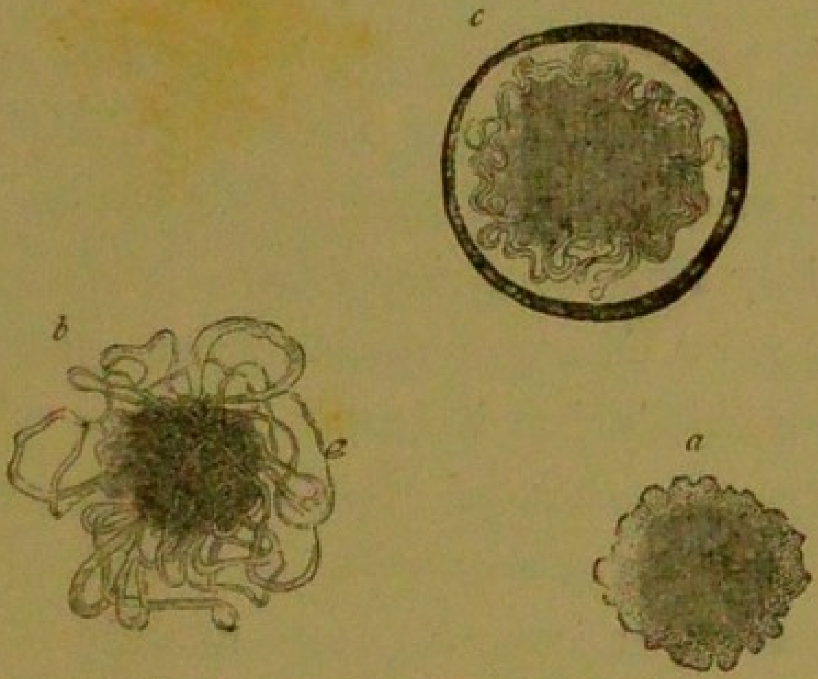

Fig. 21.—Colonies of *bacillus violaceus:* (*a*) colony in the depth of the gelatine; (*b*) thread-like ramifications extending in all directions; (*c*) later stage of development when the liquefaction of the gelatine has commenced. (*After Grace C. Frankland.*)

each successive decade of the past half-century, was naturally soon impressed into the service of those who sought to explain the empirical fact that these particular diseases are frequently communicated by water. It is significant that these views concerning the propagation of cholera and typhoid, and the importance to be, therefore, attached to drinking-water in connection with public health, are mainly English in origin, and were for many years unflinchingly preached and practised by English sanitary authorities, at a time when the germ theory of disease was a speculation, and not established as it now is.

The germ theory of disease having thus so early become interwoven with the consideration of potable waters, it is easy to understand with what eager interest the vigorous development in our knowledge of micro-organisms in general, and of their connection with disease in particular, which has taken place within the past fifteen years, has been watched by those who have had to devote much attention to the sanitary aspects of water-supply.

IV.

USEFUL MICRO-ORGANISMS.

We must now turn our attention to some of the works of utility upon which many varieties of micro-organisms are continually engaged without rest day and night, and upon the fruit of which labours man is deeply dependent.

Amongst the vast array of chemical changes or chemical reactions, as we generally call them, which constitute the great science of chemistry, there is probably none which is so well known to the general public, and certainly none which enjoys a more ancient reputation and history, than the conversion of sugar into alcohol. There is hardly any race of men so primitive that they have not discovered the method of effecting this change, for to whatever part of the world we turn our attention, we find that in some shape or other a fermented liquid, or in other words an alcoholic liquid, is the favourite beverage of man.

But although the production of this substance has been known from the most remote times, the nature of this change from sugar to alcohol is still one of the obscurest in the whole of chemical science, is still one that the ingenuity and resource of man has been unable to imitate.

It was first found some fifty years ago by Cagniard Latour and Schwann, that the mysterious substance known to brewers as *yeast* or *barm* was really composed of a vast number of minute oval particles endowed with the powers of growth and multiplication, and, therefore, undoubtedly living. This substance, which was vulgarly known as yeast, having attracted the attention of scientific men, was in course of time rechristened, and received the more imposing, though less generally intelligible, title of *saccharomyces cerevisiæ.* Comparatively little further progress was made in our knowledge of these minute forms of life until the alcoholic fermentation was submitted to the most exhaustive investigation by Pasteur, who spent many of the best years of his life in the study of these small oval particles of yeast. These memorable researches of Pasteur showed that much of the success in brewing depends upon the use of pure yeast, free from other organisms; whilst by the more recent investigations of Christian Hansen, of Copenhagen, it has been found that it is not only necessary to employ yeast free from other organisms, but that for every particular kind of beer a particular kind of yeast and no other must be applied. This has led to what is known as the pure cultivation of yeasts for brewing purposes. This cultivation of pure yeasts requires the very greatest technical skill and scientific accuracy, and several special laboratories have been established for the purpose on the Continent. It is a most impressive sight to witness the careful preparation of these various pure yeast-growths, each yeast possessing particular virtues of its own which it will carry to any quarter of the globe to which it may be transmitted from the laboratory; for the advantages of these pure yeasts over the old haphazard mixtures are now becoming so widely recognized that numerous breweries in all parts of the world draw their supplies from these continental laboratories. These yeasts are divisible into two great classes for practical purposes, according as they grow upon the surface or at the bottom of the liquid in which they are inducing the alcoholic fermentation. The *Top-Fermentation Yeasts* are those exclusively employed in the production of English, Scotch, and North German beers; whilst the *Bottom-Fermentation Yeasts* are those used for the brewing of those South German and Austrian beverages which have in recent years acquired such great popularity in almost every part of the world as Lager Beer and Pilsen, and which are produced on such an enormous scale at Munich, and almost throughout the whole of Bavaria.

The two following diagrams serve to illustrate broadly the differences between these two kinds of yeasts.

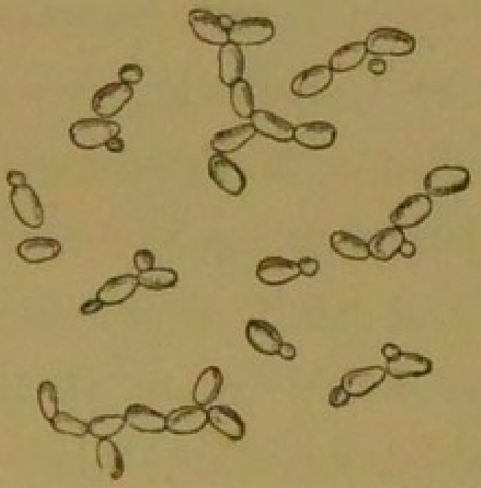

Fig. 22.—Upper Yeast.

Fig. 23.—Under Yeast.

But a mere microscopic inspection of this kind is quite inadequate to really distinguish between the different varieties of yeast, and it is to Hansen that the credit is due of having devised a series of tests to which they may be submitted in order that their individual characters may be ascertained.

Now, the principal consumer of alcohol is, as we know, Man; for the lower animals evince no affinity for alcohol in any of its various forms, and to plant-life alcohol is actually fatal when administered in any considerable quantity. There is, however, one exception to this general antipathy manifested by the lower creation to this material which exercises such a mesmeric influence over the human race. We find, indeed, amongst the lowest of living organisms, amongst the bacteria, a single form the capacity for alcohol of which not only equals but far exceeds that of the most confirmed inebriate. The organism possessing this truly human instinct is a minute body presenting, when magnified 1000 times under the microscope, the appearance which you see in the following figure.

The existence and properties of this organism were first revealed by Pasteur, who gave it the name of *Mycoderma aceti*. It has the property of taking up its abode in alcoholic liquids of moderate

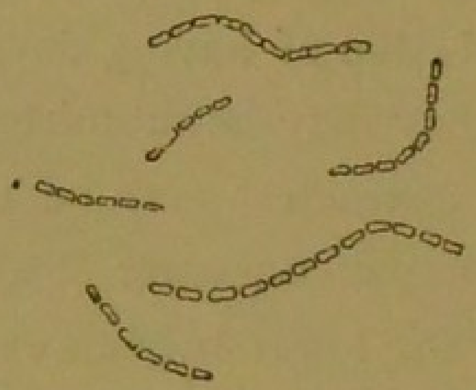

Fig. 24.—Vinegar Organism.

strength, and of there multiplying with extraordinary rapidity, consuming the alcohol and transforming it at a fabulous rate into that intensely sour substance known as acetic acid or vinegar. This organism is not, however, an undiscriminating dipsomaniac, grasping recklessly at anything which is alcoholic; but on the contrary it refuses absolutely to have anything to do with spirituous liquids which are above a certain strength, viz., anything containing much above 10% of alcohol. That the wisdom of such an aversion to the stronger alcoholic drinks could be developed in mankind were indeed a consummation devoutly to be wished!

Through the agency of this small organism the wine prepared according to the most approved methods from the choicest vintages, matured for years in the best cellars in cask and bottle, is converted in the course of a few hours into a sour liquid, and its value reduced from shillings to as many pence.

It is, however, with regard to the bacteria connected with other industries than those of alcoholic fermentation that our knowledge has particularly advanced during the last few years. Thus some of the chief phenomena in agriculture have recently received a most remarkable elucidation through the study of bacteria. Perhaps the most striking changes which micro-organisms bring about are those in connection with animal and vegetable matter. Under ordinary circumstances these animal and vegetable matters undergo putrefaction and decay, or, in common parlance, they "go bad." Formerly it was supposed that this "going bad" was an inherent property of such substances, but it is now known to be entirely due to the action of various micro-organisms; and if due precautions be taken to exclude these micro-organisms, vegetable and animal substances are found to be almost quite permanent. Therefore, but for the agency of bacteria, the surface of the earth would be covered with the remains of plants and animals undergoing but little more change than the stones and other mineral ingredients of which the earth's crust is composed. But under these circumstances life, as we now know it upon the surface of the globe, would soon come to an end, for it is by the decomposition of refuse animal and vegetable matter in the ground that the fertility of the soil is maintained, and in the absence of this decomposition, which, as I have said, can only be effected by the agency of micro-organisms, the most fertile land would soon become a barren waste, incapable of supporting plant-life; and upon the extinction of the latter the cessation of animal-life would rapidly follow as a necessary consequence.

Thus, whilst the animal is directly dependent upon the vegetable kingdom, the latter owes its sustenance to the products elaborated by micro-organisms from refuse animal and vegetable matters; and if one link, although at first sight perhaps the least important one in the chain, be broken, the whole mechanism, with its wonderful cycle of changes, must necessarily collapse.

Scientific agriculturists are generally agreed that one of the most important plant-foods in the soil is *Nitric acid;* indeed they inform us that if a soil were utterly destitute of this material, it would be incapable of growing the barest pretence of a crop *either of corn, or of roots, or of grass,* even if the soil were in other respects of the most superb texture, however favourably it might be situated, however well drained, tilled, and supplied with the purely mineral ingredients of plant-food, such as *potash, lime, and phosphoric acid.*

Yet notwithstanding the commanding importance of this substance, nitric acid, to vegetation, it is present in ordinary fertile soils in but little more than the most minute doses. These facts are gathered from the important experiments which have during the past half-century been going on at Rothamsted, under the direction of Sir John Lawes and Dr. Gilbert. Now the cause of such minute quantities only of nitric acid being found in soils is due partly to this material being washed away by the rain, and partly to its being so eagerly taken up by plants for the purposes of nutrition; for it has long been known that by suitable means the quantity can be enormously increased if no vegetation is maintained, and the ground properly protected from rain. The soil in fact, under ordinary circumstances, continuously generates this nitric acid from the various nitrogenous manures which are applied to it, and it is in the form of nitric acid that the nitrogen of manures principally gains access as nutriment to the plant.

It was in the year 1877 that two French chemists, Schloesing and Müntz, showed that this power of soils to convert the nitrogen of nitrogenous substances into nitric acid was due to low forms of life—to micro-organisms or bacteria. Since that time many interesting facts have been elucidated by Warington, Munro, and others, but the particular organism responsible for this important reaction succeeded in eluding all attempts at its identification and isolation. The details of the many efforts which were made in this direction by different investigators would occupy too much time to describe. Suffice it to say that in

1890 this organism was at length isolated, and that about a month after I had succeeded in separating it out, Winogradsky, who had also been working at the same subject, announced his discovery of a very similar if not identical nitrifying organism.

Fig. 25.—Nitrifying organism (*Percy Frankland*) possessing the power of converting ammonia into nitrous acid.

The difficulty of obtaining this organism in a state of purity was enhanced by its inability to grow upon any of the usual culture-media employed, requiring for its growth an entire absence of all organic matter. In fact I have kept it alive in purely mineral solutions for upwards of four years. It has, moreover, been proved by Winogradsky, beyond all reasonable doubt, that the nitrifying organisms not only flourish, but multiply, and actually build up living protoplasm in a solution from which organic matter has been most rigorously excluded. If these experiments are correct, they are subversive of one of the fundamental principles of vegetable physiclogy, which denies to all living structures, save those of green plants alone, the power of building up protoplasm from such simple materials.

But these discoveries had not completely unravelled the problem of nitrification, for the organisms thus separated out were found to possess only the property of *converting ammonia into nitrous and not into nitric acid.* The nitrous acid is an intermediate body which curiously is rarely found excepting in very minute quantities in soil, and the conversion of ammonia into nitrous acid is accomplished with far more difficulty by *purely chemical means* than the conversion of the latter into nitric acid, which can be done in the laboratory with the greatest facility.

But how then is the nitric acid found in the soil produced, when these organisms yield only nitrous acid? At the time when I found that the organism which I had isolated produced only nitrous acid, I pointed out that it was doubtless explicable on one of two hypotheses. (1) That nitrous and nitric acids are produced by totally distinct organisms, or (2) that the same organism produces the one or the other according to the conditions under which it is growing.

More recent researches of Warington and Winogradsky have shown that the first of these two alternative hypotheses is the correct one, for by making cultivations of soil in a solution containing nitrous acid and no ammonia, a micro-organism has been isolated which possesses the power of converting nitrous acid into nitric acid, but has no power of attacking ammonia.

The process of nitrification in the soil now becomes intelligible in its entirety; it is the work of two independent organisms, the first of which converts ammonia into nitrous acid, whilst the second transforms into nitric acid the nitrous acid produced by the first.

Both of these bacteria refuse to grow on the ordinary solid cultivating media employed by bacteriologists; this difficulty has, however, been overcome in a most ingenious manner, originally devised by Professor Kühne, in which the solid medium is composed of mineral ingredients, the jelly-like consistency being obtained by means of Silica. By means of this Silica-jelly colonies of the nitrifying organism have been obtained, as in the case of other micro-organisms with ordinary gelatine-peptone.

There is a point in connection with the distribution of nitric acid in nature which is exceedingly remarkable, and which forces itself upon the attention of every student of the process of nitrification. Although nitric acid is generally so scantily present in the soil, there is one notable exception to this rule, for in the rainless districts of Chili and Peru there are found immense deposits of nitrate of soda, or Chili-saltpetre as it is called, which would appear to represent the result of a gigantic nitrification process in some previous period of the earth's history. The vast quantities of this material which occur in these regions of South America can be gathered from the fact that its exportation has for years been going on at the rate indicated by the following figures:—During the first six months of 1890 there were brought to

The United Kingdom ...	90,000 tons
The European Continent	480,000 „

From the presence of such altogether enormous quantities, one is almost tempted to hazard the suggestion that in this particular region of the earth, under some special circumstances of which we know nothing, the nitrifying organisms must have been endowed then and there with very much greater power than they possess to-day, and it is particularly noteworthy that in a recent examination of soils from nearly all parts of the earth, one coming from Quito, and therefore not far distant from these nitrate-fields, was found to possess the power of nitrification in a degree far beyond that exhibited by any other soil hitherto experimented with. Is it not possible, perhaps, that we have in these vigorous nitrifying organisms of the soil of Quito the not altogether unworthy descendants of that Cyclopean race of nitrifying bacteria which must have built up the nitrate wealth of Chili and Peru, and which thus countless ages ago founded the fortunes of our Nitrate Kings of to-day?

But whilst the study of the bacteria giving rise to nitrification has thus led to the subversion of what was regarded as a firmly-established principle of vegetable physiology (viz. *the incapacity of any* *but green plants to utilize carbonic acid in the elaboration of protoplasm*), the same science has received another shock of perhaps equal if not greater violence through researches which have been carried on with other micro-organisms flourishing in the soil.

For nearly a century past agricultural chemists and vegetable physiologists have been debating as to whether the free nitrogen of our atmosphere can be assimilated or utilized as food by plants. This question was answered in the negative by Boussingault about fifty years since; the problem was again attacked by Lawes, Gilbert, and Pugh about thirty years ago, and *their* verdict was also to the same effect. In the course, however, of their continuous experiments on crops, Lawes and Gilbert have frequently pointed out that whilst the nitrogen in most crops can be accounted for by the combined nitrogen supplied to the land in the form of manures and in rain-water, yet in the case of particular *leguminous* crops, such as peas, beans, vetches, and the like, there is an excess of nitrogen which cannot be accounted for as being derived from these obvious sources.

The origin of this excess of nitrogen in these particular crops they admitted could not be explained by any of the orthodox canons of the vegetable physiology of the time. The whole question of the fixation of atmospheric nitrogen by plants was again raised in 1876 by a very radical philosopher, in the person of M. Berthelot, whilst the most conclusive experiments

Fig. 26.—Roots of Sainfoin. (*Lawes and Gilbert.*) (*a*) Nodules.

were made on this subject by two German investigators, Professor Hellriegel and Dr. Wilfarth, who have not only shown that this excess of nitrogen in leguminous crops is obtained from the atmosphere, but also that this assimilation of free nitrogen is dependent upon the presence of certain bacteria flourishing in and around the roots of these plants, for when these same plants are cultivated in sterile

Fig. 27.—Roots of Sainfoin. (*Lawes and Gilbert.*) (*a*) Nodules.

soil the fixation of atmospheric nitrogen does not take place. Moreover, the presence of these microbes in the soil occasions the formation of

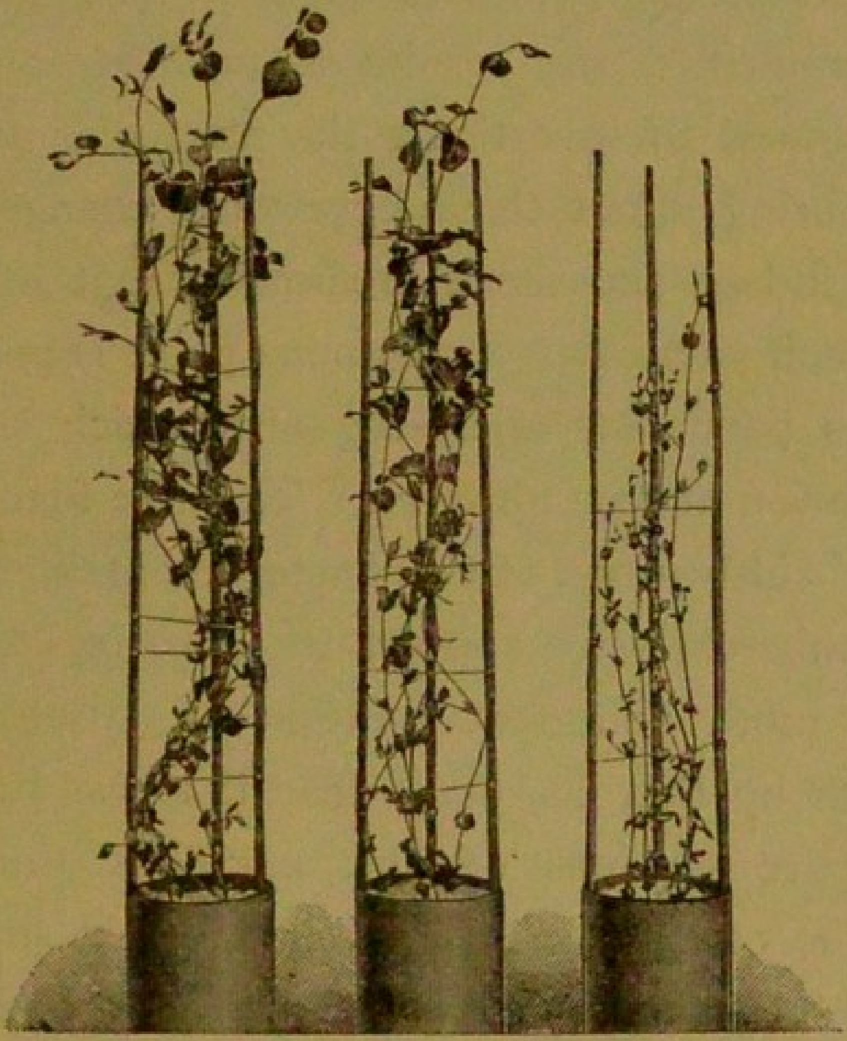

Fig. 28.—Pea-Plants. (*After Nobbe.*) No. 1. Inoculated with pure cultures from tubercles of pea. No. 2. Inoculated with pure cultures from tubercles of lupin. No. 3. Inoculated with pure cultures from tubercles of robinia. The inoculations were all made on August 14th, 1890, and the plants photographed on October 9th, 1890.

peculiar swellings or tuberosities on the roots of these plants, and these tuberosities which are not formed in sterile soil are found to be remarkably rich in nitrogen and swarming with bacteria.

Extremely important and instructive are the experiments of Professor Nobbe, who has not only confirmed the results mentioned, but has endeavoured to investigate the particular bacteria which bring about these important changes, and he has indeed succeeded in showing that in many cases each particular leguminous plant is provided with its particular micro-organism which leads to the fixation of free nitrogen. Thus, he found that if pure cultivations of the bacteria obtained from a pea-tubercle were applied to a pea-plant, there was a more abundant fixation of atmospheric nitrogen by this pea-plant than if it was supplied with pure cultures of the microbes from the tubercles of a lupin or a robinia, whilst similarly the robinia was more beneficially affected by the application of pure cultures from robinia-tubercles than by those from either pea-tubercles or lupin-tubercles.

To micro-organisms again then we must ascribe the accomplishment of this highly important chemical change going on in the soil, although it

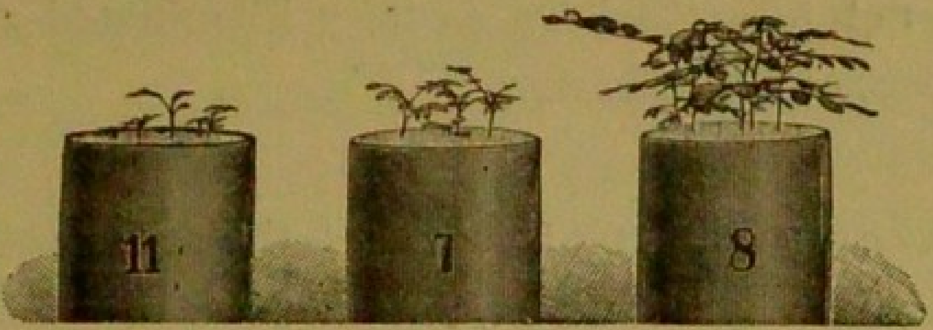

Fig. 29.—Robinia. (*After Nobbe.*) No. 11. Uninoculated. No. 7. Inoculated with pure cultures from pea-tubercles. No. 8. Inoculated with pure cultures from robinia-tubercles. The inoculations were made on June 27th, 1890; the plants were photographed on August 5th, 1890.

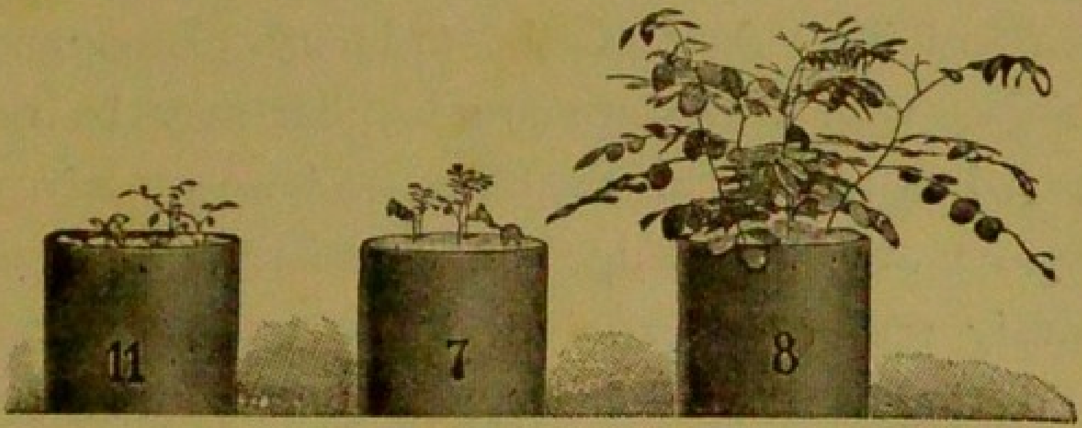

Fig. 30.—Photographed on August 21st, 1890.

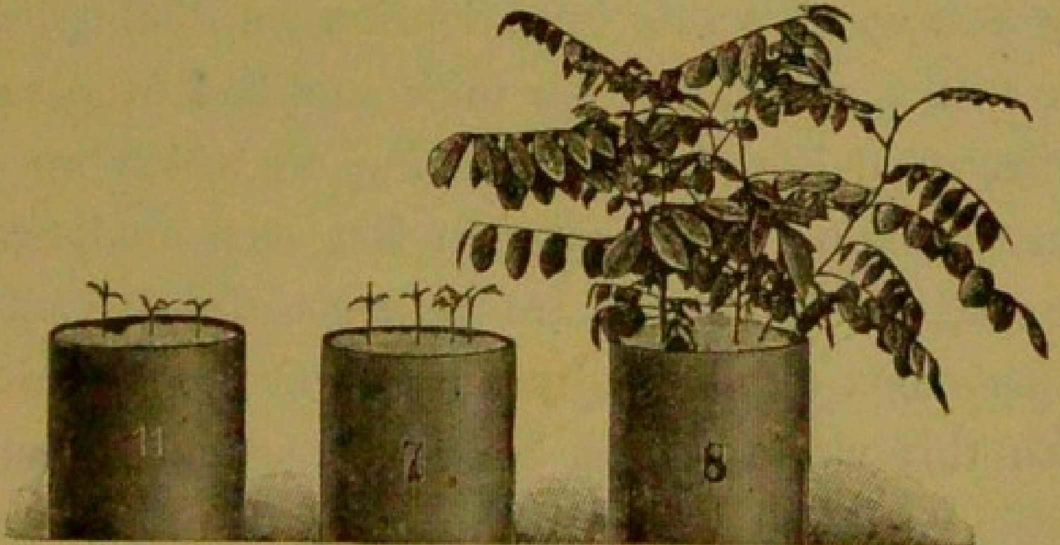

Fig. 31.—Photographed on October 3rd, 1890.

has not hitherto been so fully illuminated as the process of Nitrification.

Before passing on to some of the other works of a less friendly character, for which we are indebted to micro-organisms, I should like to draw your attention to a very remarkable and important characteristic possessed in general by these lowly forms of life. Any of the ordinary plants and animals with which we are familiar may be regarded as analytical machines, and we ourselves, without any knowledge of chemistry, are constantly performing analytical tests; thus we can all distinguish between sugar and salt by the taste, between ammonia and vinegar by the smell, whilst by a more elaborate investigation we distinguish, for instance, between the milk supplied from two different dairies by ascertaining on which we or our children thrive best. In fact such analytical or selective operations are amongst the first vital phenomena exhibited by an organism on coming into this world. It is, however, particularly surprising to find this analytical or distinguishing capacity developed in an extraordinarily high degree amongst micro-organisms. From the power which we have seen that some possess of flourishing on the extremely thin diet to be found in distilled water, we should be rather disposed to think that caprice would be the very last failing with which they would be chargeable. As a matter of fact, however, the perfectly unfathomable and inscrutable caprice of these minute creatures is amongst the first things with which the student of bacteriological phenomena becomes impressed. Let me call your attention to a striking example of this which I have recently investigated.

Here are two substances which have the greatest similarity—

	Mannite.	*Dulcite.*
Occurrence	numerous plant-juices	ditto, but less frequently.
Taste	sweet	ditto, but less so.
Melts	166° C.	188° C.
Crystalline form	large rhombic prisms	large monoclinic prisms.

Now, not only do these two substances possess such a strong external resemblance to each other, but in their chemical behaviour also they are so closely allied that one formula has to do duty for both of them; so slight is the difference in the manner in which their component atoms are arranged, that chemists have not yet been able with certainty to ascertain in what that difference consists. Under these circumstances it would have been anticipated that bacteria would be quite indifferent as to which of these two substances was presented to them, and that they would regard either both or neither as acceptable. But such is by no means the case; some micro-organisms, like ordinary yeast, have *no action upon either*, whilst *others will attack both*, and *others will attack mannite, leaving dulcite untouched*, while representatives of a fourth possible class, *which would act upon dulcite but not upon mannite*, are as yet undiscovered.

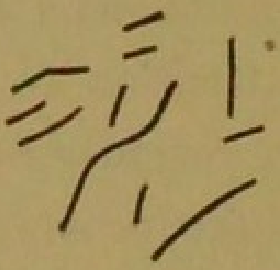

Fig. 32.—*Bacillus ethaceticus.* (*After Grace C. Frankland.*)

The accompanying figure shows an organism which I discovered to be capable of acting upon mannite but not on dulcite. I have christened it *Bacillus ethaceticus*, because of the substances which it yields during its fermentative action on various materials.

Again, more recently I have, in conjunction with Mr. Frew, succeeded in obtaining a micro-organism which decomposes both mannite and dulcite, and which has received, also on account of the products which it yields, the somewhat unwieldy title of *Bacillus ethacetosuccinicus.*

But these are by no means the ultimate limits to which the selective or discriminating powers of micro-organisms can be pushed, for although mannite and dulcite are extremely similar substances, they art not chemically identical. We are acquainted, however, with substances which, though chemically identical, are different in respect of certain physical properties only, and are

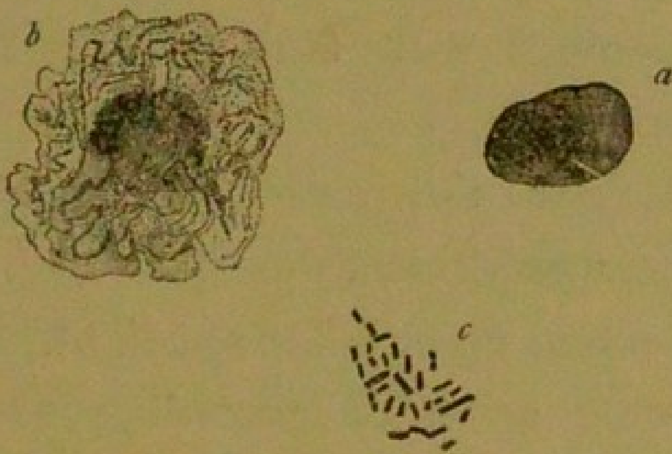

Fig. 33.—*Bacillus ethacetosuccinicus.* (*a*) Colony in the depth of the gelatine. (*b*) Colony on the surface. (*c*) Individual bacilli. (*After Grace C. Frankland.*)

hence known as *Physical Isomers.* It is in explanation of this physical isomerism that one of the most beautiful of chemical theories was propounded by Lebel and Van 't Hoff, in 1874, and remains unsupplanted to the present day.

This theory depends upon taking into consideration the dissymmetry of the molecule, which is occasioned by the presence in it of a carbon-atom, which is combined with four different atoms or groups of atoms.

This molecular dissymmetry is specially exhibited in the crystalline form of such substances, and in their action upon polarized light.

The molecule arranged according to the one pattern has the property of turning the plane of polarization in one direction, whilst the molecule arranged according to the other pattern has invariably the property of turning the plane through precisely the same angle in the opposite direction. The molecular dissymmetry ceases when two such molecules combine together, the resulting molecule having no action on polarized light at all.

The interest of these phenomena in connection with micro-organisms lies in the fact that they are sometimes possessed of the power of discriminating between these physical isomers. Although this remarkable property was demonstrated years ago by Pasteur in respect of the tartaric acids, it has only comparatively rarely been taken advantage of. Recently, however, chemical science has been enriched in several instances by successfully directing the energies of micro-organisms in such work of discrimination.

During the past few years no chemical researches have commanded more interest, both on account of their theoretic importance and the fertility of resource exhibited in their execution, than those of Emil Fischer, which have led to the artificial preparation in the laboratory of several of the various forms of sugar occurring in nature, as well as of other sugars not hitherto discovered amongst the products of the animal or vegetable kingdoms. The ordinary natural sugars are all bodies with dissymmetric molecules, powerfully affecting the beam of polarized light, but when prepared artificially they are without action on polarized light, because in the artificial product the left-handed and right-handed molecules are present in equal numbers, the molecules of the one neutralizing the molecules of the other, and thus giving rise to a mixture which does not affect the polarized beam either way. By the action of micro-organisms, however, on such an inactive mixture, the one set of molecules are searched out by the microbes and decomposed, leaving the other set of molecules untouched, and the latter now exhibit their specific action on polarized light, an active sugar being thus obtained.

The most suitable micro-organisms to let loose, so to speak, on such an inactive mixture of sugar-molecules, are those of brewers' yeast, which decompose the sugar-molecules with formation of alcohol and carbonic anhydride. Their action on these inactive artificial sugars of Fischer's is particularly noteworthy.

One of the principal artificial sugars prepared by Fischer is called *fructose;* it is inactive, but consists of an equal number of molecules of oppositely active sugars called *laevulose.*

One set of these laevulose-molecules turns the plane of polarization to the right, and we may call them *right-handed laevulose*, whilst the other set of *laevulose-molecules* turns the plane of polarization to the left, and we may call them *left-handed laevulose.*

The left-handed laevulose occurs in nature, whilst the right-handed laevulose, as far as we know, does not. Now, on putting brewers' yeast into a solution of the fructose, the yeast-organisms attack the left-handed laevulose-molecules and convert them into alcohol and carbonic anhydride, whilst the right-handed laevulose is left undisturbed. The yeast-organisms thus attack that particular form of laevulose of which their ancestors can have had experience in the past, whilst they leave untouched the right-handed laevulose-molecules, which, being a new creation of the laboratory, they have no hereditary instinct or capacity to deal with.

This selective power is possessed also by other forms of micro-organisms besides the yeasts, which are indeed only suitable for the separatory decomposition of sugars, and by means of bacterial forms a much greater variety of substances can be attacked in this manner. Thus I have lately found that glyceric acid can be decomposed by the *B. ethaceticus* already referred to.

This glyceric acid should, according to Lebel and Van 't Hoff's theory, be capable of existing in two physically isomeric forms.

The ordinary glyceric acid known to chemists is, however, quite inactive to polarized light, and must consist, therefore, of a combination in equal molecules of a right-handed and left-handed glyceric acid. Now when the *B. ethaceticus* is put into a suitable solution of the calcium salt of this glyceric acid, it multiplies abundantly, and completely consumes the right-handed molecules of the salt, but leaves the left-handed molecules entirely intact, and thus I was able to obtain for the first time a powerfully active glyceric acid.

But I must not allow the fascination attaching to the idiosyncracies of micro-organisms to encroach too much upon our time, and must omit many other interesting and beautiful instances of this selective action, on account of their being possibly too technical in their character to excite general interest. I cannot, however, resist in conclusion drawing your attention to the manner in which these idiosyncracies may be changed, and the way in which their individuality may be affected by judicious treatment.

Although micro-organisms are becoming more and more indispensable re-agents in the chemical laboratory, essential as they are for the production of many bodies, it is always necessary to bear in mind that by virtue of their vitality their nature is infinitely more complex than that of any inanimate chemicals which we are accustomed to employ. In a chemically pure substance we believe that one molecule is just like another, and hence we expect perfect uniformity of behaviour in the molecules of such a pure substance, under prescribed conditions. In a pure cultivation of a particular species of a micro-organism, however, we must not expect such rigid uniformity of behaviour from each of the individual organisms making up such a cultivation, for there may be and frequently are great differences amongst them, in fact each member of such a pure culture is endowed with a more or less marked individuality of its own, and these possible variations have to be taken into consideration by those who wish to turn their energies to account. In fact, experimenting with micro-organisms partakes rather of the nature of legislating for a community than of directing the inanimate energies of chemical molecules.

Thus frequently the past history of a group of micro-organisms has to be taken into account in dealing with them, for their tendencies may have become greatly modified by the experiences of their ancestors. In support of this I will mention the following instance, which recently came under my observation. Some time ago I came upon a bacillus which has the property of fermenting calcium citrate, and I have found that it can go on exerting this power for years. But if it is plate-cultivated, or in other words if we induce it to grow on gelatine-peptone, and take one of the resulting colonies and transfer it to a sterile solution of calcium citrate, identical in every way to that which before it readily fermented, *it invariably fails to set up any fermentation*, the bacillus having thus, by mere passage through the gelatine medium, lost its power to produce this effect. If, however, we take another similar colony and put it into a solution of broth containing calcium citrate, fermentation takes place ; on now inoculating from this to a weaker solution of broth containing calcium citrate this also is put into fermentation, and by proceeding in this manner we may ultimately set up fermentation in a calcium citrate solution, which absolutely refused to be fermented when the bacilli were taken directly from the gelatine plate.

Phenomena of this kind clearly indicate that there may be around us numerous forms of micro-organisms, of the potentiality of which we are still quite ignorant; thus, if we were only acquainted with the bacilli I have just referred to from gelatine cultures, we should be quite unaware of their power to excite this fermentation of calcium citrate, which we have only been enabled to bring about by pursuing the complicated system of cultivation I have described. It is surely exceedingly probable, therefore, that many of the micro-organisms with which we are already acquainted may be possessed of numerous important properties which are lying dormant until brought into activity by suitable cultivation.

The power of modifying the characters of bacteria by cultivation is, I venture to think, of the highest importance in connection with the problems of evolution, for in these lowly forms of life in which, under favourable circumstances, generation succeeds generation in a period of as little as twenty minutes, it should be possible, through the agency of selection, to effect metamorphoses, both of morphology and physiology, which would take ages in the case of more highly-organized beings to bring about.

We hear much from the enthusiastic apostles of education about the possibility of altering the human race through a suitable course of training, but even the most sanguine of these theorists cannot promise that any striking changes will be effected within several generations, so that such predictions cannot be tested until long after these reformers have passed away.

In the case of micro-organisms, however, we can study the effect of educational systems consequentially pursued through thousands of generations within even that short span of life which is allotted to us here.

V.

MALIGNANT MICRO-ORGANISMS.

THE properties of micro-organisms which we have hitherto considered have been harmless or even beneficial to man; there are, however, a number of varieties of microbes whose behaviour is anything but amiable, and whose dangerous character has brought such discredit upon the entire class of micro-organisms, that the virtues of some are often overlooked, and their usefulness forgotten, owing to the terror and dismay which their harmful brethren inspire. For, as you know, some of them exist as parasites on the higher organisms, including man himself; of these higher organisms they frequently cause the degeneration and death, producing the severest diseases amongst animals high and low, and threatening mankind with the most murderous plagues and epidemics. We have already learnt to call such mischievous varieties *Pathogenic*, whilst we usually call the diseases to which they give rise *Zymotic*, in consequence of their course presenting more or less resemblance to a process of fermentation. Thus, the manner in which infectious diseases are communicated, the continuous propagation of the infectious principle through a long series of individuals, the occasional transportation of the infection to long distances; again, the period of incubation, and the typical manner in which these diseases run their course, all are circumstances which have long and irresistibly impressed observers with the organized or living nature of the exciting cause.

In fact, although we are firmly convinced that all the zymotic diseases, such as cholera, scarlet fever, typhoid fever, measles, small-pox, diphtheria, hydrophobia, etc., are due to the activity of micro-organisms, yet it is in comparatively few cases that particular micro-organisms have been conclusively proved to be the cause of a particular disease.

We will now turn our attention to some of the cases in which diseases have been traced to the agency of micro-organisms.

The disease which of all others has been most thoroughly studied in this respect is one which we have already had occasion to mention, viz. *Woolsorters' Disease*, or *Malignant Pustule;* it is much dreaded also by farmers, owing to the ravages which it makes among stock, the particular disease in cattle being known as *Splenic Fever.* It was in the year 1850 that two French doctors, Rayer and Davaine, first stated that they had found small, rod-like bodies in the blood of subjects whose death was due to anthrax. These are the words in which they announce this most important discovery :—" On trouve dans le sang de petits corps filiformes ayant environ le double en longueur du globule sanguin. Ces petits corps n'offrent point de mouvement spontané."

But here the matter rested, for no further notice was taken of these "petits corps filiformes," and this indifference lasted for upwards of twelve years, until in 1863 attention was again directed to these mysterious forms, in consequence of the public interest which was attracted by the researches of Pasteur.

It was not until the year 1877 that Pasteur, in a memoir read before the Academy of Sciences of Paris, demonstrated beyond all doubt that these small, rod-like bodies, first observed by Davaine and Rayer in 1850, were the sole exciting cause of anthrax. Since that time our acquaintance with these bacilli has been greatly extended by Robert Koch and others, and our knowledge of their life-history confirmed by the most exhaustive and careful investigations. These researches are still continuing, and we shall see later on to what wonderful results they have led in combating the symptoms to which their presence gives rise.

But we will now examine a little more in detail how this disease is communicable from animal to man.

If the blood taken from any part of an animal just dead of anthrax be microscopically examined, it will be found to be teeming with these bacillar forms. Now, if the smallest quantity of such blood is introduced into the tissues of another animal capable of taking the disease, the inoculated animal becomes infected, and almost certainly succumbs; and if now the blood of this second victim be similarly examined, this also will be found densely populated with the same bacilli. We thus see that the disease is accompanied by the enormous multiplication of the micro-organism within the system of the victim, and that the disease may be indefinitely communicated from one animal to another.

But we may also cause this bacillus to grow and multiply abundantly outside the animal system altogether, or, as we term it, *cultivate the organism in an artificial medium.* Thus, if we take on the point of a needle the minutest trace of the blood of an animal dead of splenic fever, and then introduce the point of the needle into any of the ordinary cultivating media, such as broth, gelatine-peptone, agar-agar,[1] or blood-serum, we shall obtain in the course of a few days an abundant growth of the anthrax bacillus, readily visible to the naked eye. I have before referred to the highly characteristic appearances to which this organism gives rise on being artificially cultivated. In the accompanying figures some of these appearances are illustrated. In Fig. 34 *a* we see one of these

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[1] Japanese isinglass, capable of remaining solid at a higher temperature than ordinary gelatine.

anthrax colonies greatly magnified, growing on the surface of a gelatine-plate. The peculiar wavy bands and whip-like projections remind us of the colonies of *bacillus subtilis.* Fig. 34 *b* represents a younger anthrax colony growing in the depth of the gelatine before it has come to the surface. Fig. 35 *a* and *b* shows the individual bacilli growing singly and in long vermiform threads; at

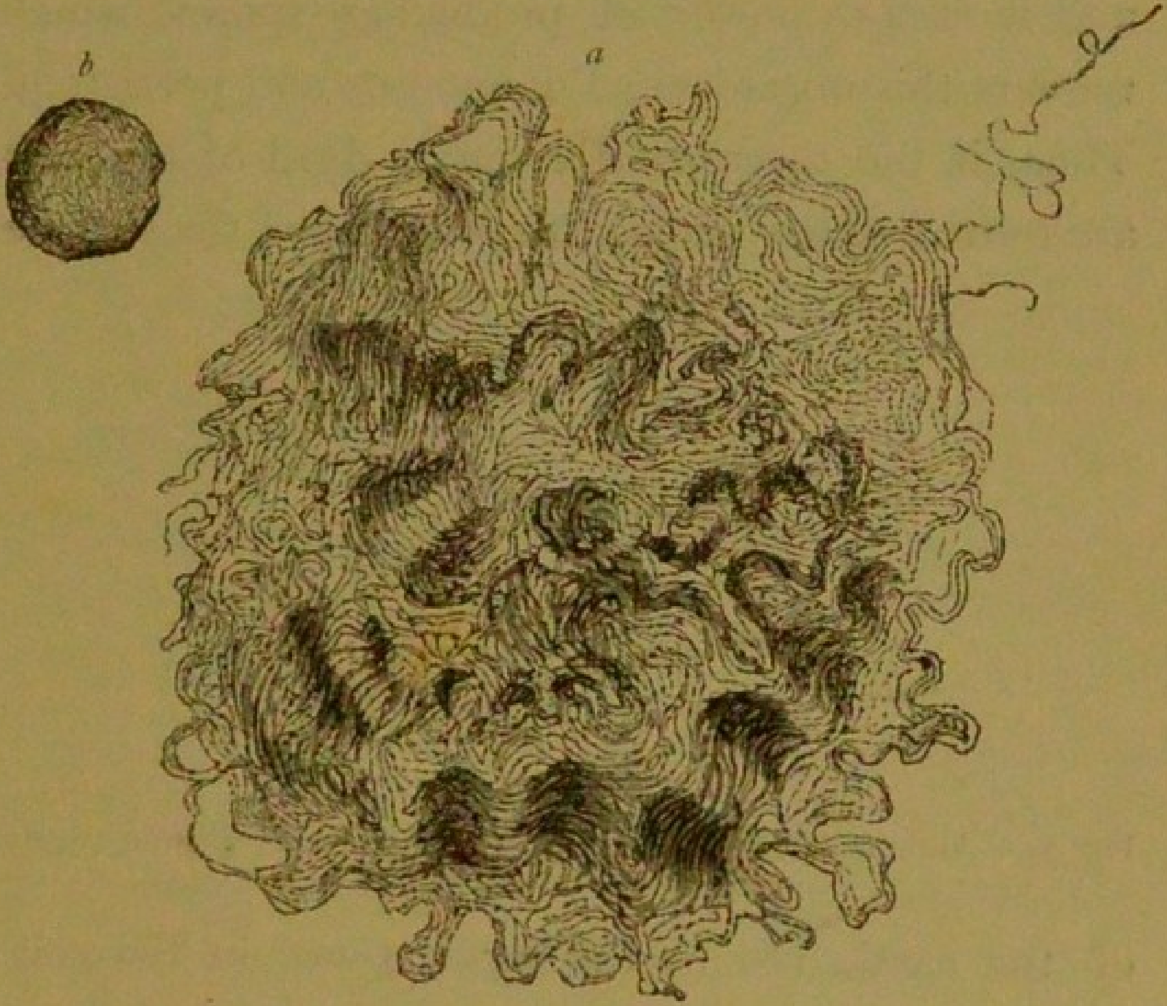

Fig. 34.—Colonies of Anthrax bacilli. (*a*) Surface colony much enlarged; (*b*) Small depth colony. (*After Flügge.*)

c spores are also visible. In the blood of the subject affected these anthrax bacilli are not able to form spores, but outside the body they give rise abundantly to these indestructible forms, and it is this power of producing spores which renders this organism so dangerous and persistent. Thus, if the carcases of animals dead of anthrax are buried, or are allowed to decay upon the surface of the earth, the bacilli form spores in the soil, and healthy animals may thus become infected by taking in the spores with their food when grazing.

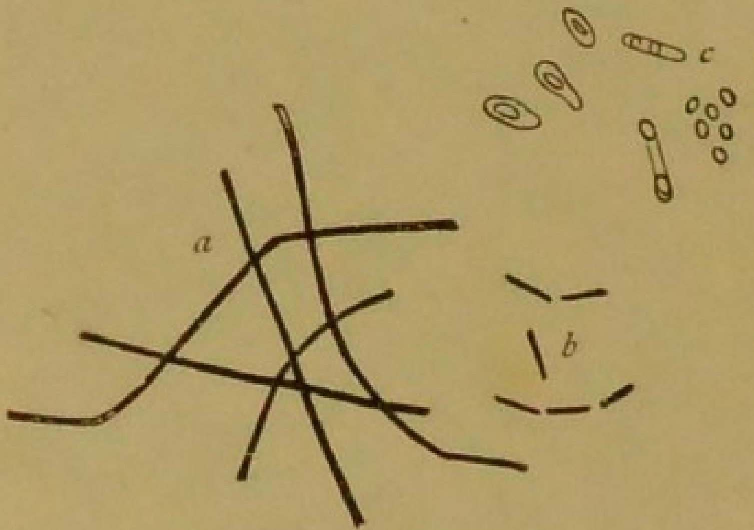

Fig. 35.—Anthrax bacilli. (*a* and *b*) individual bacilli growing singly and in threads; (*c*) spores.

Again, the skins of animals which have died of anthrax not unfrequently pass into commerce, and often prove fatal to the tanners and woolsorters who handle them, even long afterwards.

From this it will be seen how necessary it is that the strictest supervision should be exercised whenever an outbreak of splenic fever takes place, and that the disposal by cremation of the carcases of the affected animals should be most rigorously enforced. Unfortunately, those most closely concerned with this disease are only too often quite ignorant of its dangers. Thus, during a recent outbreak of this disease in a rural district of England, the butcher to whom the slaughtering of the affected animals was intrusted, was quite unaware of the dangerous task upon which he was engaged, and only had his ignorance enlightened by himself falling a victim to the disease.

We must now turn to another disease which is far more common in man than anthrax, but which, like anthrax, is also caused by a micro-organism—in this case a micrococcus, not a bacillus. The micrococci of this disease—erysipelas—hang together in chains, and are therefore known as streptococci (Fig. 36).

These organisms can be easily cultivated in artificial media outside the body, and there can be no doubt that the organism is the cause of the disease, for erysipelas has actually been produced in man by intentionally inoculating these cultivations of the streptococcus, with the result that typical erysipelas was produced. I should mention that these experiments upon human beings were performed, not merely for the purpose of scientific inquiry, but also for the benefit of the persons inoculated, who were suffering from malignant tumours, which are sometimes alleviated by an attack of erysipelas. Owing to these experiments, therefore, we are able with confidence to affirm that this particular organism is not only capable of setting up erysipelas in animals but also in man.

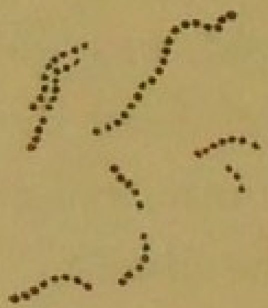

Fig. 36.—Streptococcus of Erysipelas.

But, undoubtedly, the greatest and most remarkable piece of work which has ever been accomplished in connecting disease with the life of micro-organisms, is the proof which has been furnished by Koch of the exciting cause of tuberculosis, one of the most familiar forms of which, pulmonary consumption, is the commonest disease in the world, and in this country the greatest enemy to human life, destroying as it does about one-fifth of our population, generally in the best and most active years of existence. This disease, we now know with absolute certainty, is caused by a minute bacillus, which is invariably found in all the varieties of tuberculous disease. By the most ingenious methods this bacillus has been cultivated in artificial media outside the body, and whenever reintroduced into the system of an animal it again produces the characteristic disease.

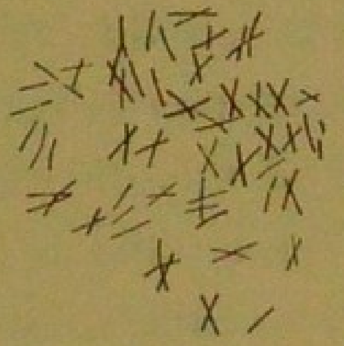

Fig. 37.—Bacillus of Tuberculosis. (*After Koch.*)

The exact manner in which this disease is communicated from one individual to another has not been actually ascertained; but when it is borne in mind that the sputum of consumptive patients almost always contains the organism, and that the bacillus is known to produce spores, it is not difficult to understand that there must be very numerous channels by which the poison may be conveyed. It would appear, however, that the mere conveyance of the organism from one person to another is not sufficient to induce the disease, but that more or less predisposition to the disease is also necessary. In the case of all the zymotic diseases, predisposition no doubt plays an important part in the process of infection, but in the case of tuberculosis this factor of predisposition appears to be of more than usual importance.

The possibility of this disease being communicated from the lower animals to man must also be borne in mind, for tuberculosis is comparatively common amongst cattle, and, as in so many other infectious diseases, milk must be viewed with suspicion as a particularly suitable medium for the conveyance of the zymotic poison.

Of immense importance is the identification within the last few years of the specific cause of tetanus, or lockjaw. Ever since Carle and Rattone proved, in the year 1884, that tetanus was a disease capable of being communicated from one subject to another, inasmuch as rabbits inoculated with pus taken from the infected part of a man who had died of tetanus also succumbed to the same disease, numerous investigators have been attracted towards this field of inquiry. Nicolaier was the first to discover that certain bacilli, widely distributed in the superficial layers of soil, were capable, when subcutaneously inoculated into mice, guinea-pigs, and rabbits, of setting up symptoms typical of tetanus from which they subsequently died. These results were confirmed by Rosenbach, but all endeavours to procure the bacillus in a pure condition, free from other micro-organisms, failed, until Kitasato, a Japanese pupil of Dr. Koch, hit upon the following ingenious plan, by which he successfully isolated the tetanus-bacillus. Kitasato took some tetanus-pus and inoculated it on to agar-agar, and kept it at a temperature of about 38° C. in an incubator; already, within the space of twenty-four hours, an abundant growth was visible, and on examining some of this under the microscope he found various micro-organisms, and amongst them bacilli which he at once recognized as those identified with tetanus by Nicolaier and others.

These bacilli form spores at the end of the rod, resembling a club in appearance. (The accompanying figure is taken from Kitasato's original paper, in which he describes the tetanus-bacillus.) They are strictly anaërobic, that is, refuse to grow in the presence of air, and hence the difficulties which arose in obtaining them in a pure state.

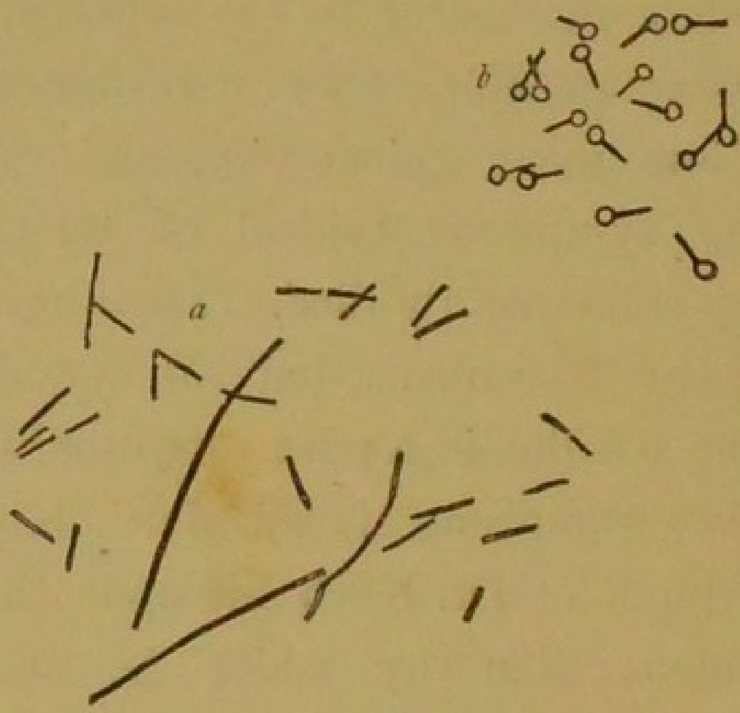

Fig. 38.—Bacilli of Tetanus or Lockjaw. (*a*) individual bacilli; (*b*) spores. (*After Kitasato.*)

Now at the end of forty-eight hours these characteristic club-shaped spore-bacilli had increased enormously, and the vessel containing the cultivation was then placed in water already heated to 80° C. and maintained at that temperature during a period of three-quarters to one hour. On examining the growth afterwards, everything was found to have been killed off except the spores, and mice inoculated with these spores died subsequently of tetanus. Kitasato was thus materially assisted in his isolation of the tetanus-bacilli by the fact that they form spores more rapidly than the other bacilli with which they are often associated, and these spores then successfully resist the high temperature which destroys the other micro-organisms. In the course of his experiments, Kitasato succeeded in procuring plate-colony-cultivations and in inducing tetanus in mice from their inoculation, and thus affording the rigid proof of the connection between this particular micro-organism and tetanus.

The spores of the tetanus-bacilli are very widely distributed, not only in garden soil, but in stables, hay-lofts, etc., and as they retain their vitality and their virulence for many months, even when dried and kept at the ordinary temperature of the air or buried in soil, they must be reckoned amongst the most determined and cruel of our microscopic foes.

Quite recently a most remarkable instance of their distribution has come under observation. A small child happened to cut its finger with an ordinary kitchen knife; its father endeavoured to stop the bleeding of the wound by binding it up with some cobwebs, a superstitious practice which is more "honoured in the breach than the observance," for the child developed nearly a month later typical symptoms of tetanus. It was proved beyond doubt that the spider's web was responsible for the mischief, for rabbits and guinea-pigs inoculated with some web taken from the same place died under particularly well-defined symptoms of tetanus. Doubtless the spores had been caught in the web, conveyed thither by some chance current of air, and had remained contentedly attached until the opportunity came for them to exercise their disastrous powers.

It has also been long known that the poisoned arrows of the savage tribes produce symptoms of tetanus in their victims, and there can be no question that in the preparation of such arrows, dipping them as they do in the mud of certain swamps and allowing them to dry in the sun, the natives unconsciously are availing themselves of the enduring and virulent properties of the tetanus-spores.

Another organism which has been very carefully studied, and whose character is yet the subject of speculation and much controversy, is the well-known comma-bacillus, identified by Koch as the responsible cause of Asiatic cholera. At present, until some other agent has been brought forward which can be shown to possess a better claim for this distinction, we must be content to recognize Koch's organism as the exciting cause of cholera, resting its title as it does upon an immense mass of evidence and most careful experimental investigation.

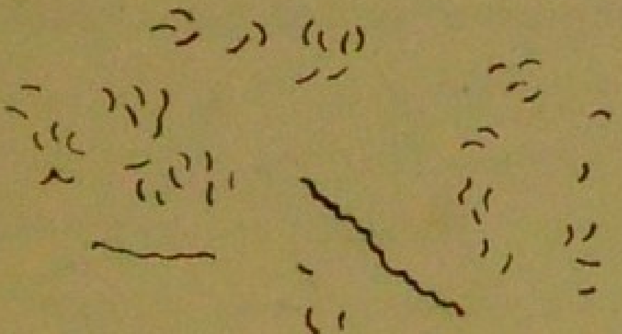

Fig. 39.—Spirilla of Asiatic Cholera, commonly known as Koch's Comma-bacilli. (*After Koch.*)

In the accompanying figure is represented a microscopic preparation of the comma-bacilli or spirilla, as they are called in consequence of the curved, corkscrew form which they frequently assume when growing in liquids.

The bacillus of typhoid fever affords another instance of a particular micro-organism being associated with a disease, but in which the difficulties of direct experimental evidence have not been overcome in such a manner as to present the same clenching proof as has been furnished in the case of the bacilli of tetanus or tuberculosis. The following figure shows the ordinary appearance of the so-called Eberth-Gaffky bacillus of typhoid fever. In a previous drawing it was represented, it will be remembered, surrounded with its flagella or organs of locomotion, which are only rendered visible by submitting the preparation to a special method of staining before microscopic examination.

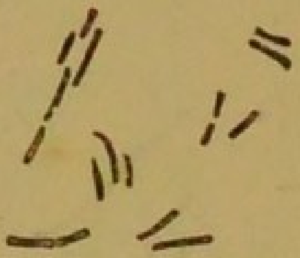

Fig. 40.—Bacillus of Typhoid Fever. (*After Migula.*)

But enough has been said to pave the way for the better appreciation of the marvellous manner in which these investigations have led, and are still leading, to the most important results in medicine. Preventive medicine, which is essentially a product of the present century, has advanced with giant strides in consequence of the rapid growth of our knowledge concerning these microscopic foes and their habits. In this great advance we have in England unfortunately lagged behind. Signs are not wanting, however, that public opinion is slowly changing in its attitude towards this branch of science, and is beginning to appreciate the importance of such beneficent investigations, which it is all but impossible at the present time to carry on in this country.

VI.

THE THEORY AND PRACTICE OF PREVENTION IN DISEASE.

WHEN we come to inquire how it is that some of these minute organisms are capable of producing these disastrous effects upon the animals in which they grow and multiply, we find that the specific symptoms to which they give rise are not, in all probability, due to the mere presence of these living particles as such, but to the fact that they elaborate within the tissues of the body certain chemical substances of a highly poisonous nature, and that these poisons, and not the microbes themselves, are the real cause of the mischief. These poisonous chemical substances may be elaborated by microbes when growing in artificial cultures outside the body; thus, when meat and other albuminous substances begin to undergo decomposition, they are frequently possessed of most intensely poisonous properties. We are all of us familiar with those remarkable instances, of which we frequently read in the papers, of a whole family being poisoned by partaking of some particular dish of meat, fish, or the like. Now, such cases are nearly invariably due to the food in question having been in an unsound condition; before being cooked, it has harboured certain micro-organisms which have produced these particular chemical substances, and whilst the microbes have been destroyed in the cooking, the poisonous character of these elaborated substances has remained unchanged by the process.

It is therefore to the absorption and circulation within the system of these intensely poisonous substances, elaborated by specific micro-organisms, that the particular disturbances of the animal system, which characterize these diseases, must be traced. The action of these pathogenic bacteria may, in fact, be compared, not to that of poisoning some one with, for instance, *nux vomica*, but it is as though the seeds of this poisonous plant could be introduced into the body of an animal, and there spring up into the plant itself, producing its poisonous materials within the system of the animal. In fact, by introducing into an animal these organized poisons like tetanus or tuberculosis, we introduce not, strictly speaking, a poison at all, but a complete poison-manufactory, or a piece of machinery which will elaborate deadly poisons out of the animal tissues and juices on which the machine is thriving and extending its dimensions. This is no fanciful picture at all, for in several cases the actual poisons produced by these parasitic micro-organisms have been both discovered and isolated. Thus Brieger has described substances which he was able to obtain from pure cultivations of the typhoid and tetanus bacilli. In the case of the typhoid growth, he isolated a material which he called typhotoxine, whilst from the products elaborated by the tetanus bacilli he procured a substance—tetanine—which, when injected into animals, produces characteristic tetanic symptoms; and tetanotoxine, likewise obtained from tetanus cultivations, in employing which he was able to induce some of the symptoms accompanying tetanus. Brieger has also isolated a very poisonous substance from decomposing mussels, and other poisonous materials have been separated from putrefying cheese, etc.

But investigation has not been allowed to stop here; scientific men have not been content with merely unravelling the cause of these terrible phenomena which are constantly going on around us, but having carefully taken the measure, so to speak, of these countless hordes antagonistic to human life, reconnoitred their strongholds, and examined the weapons with which they fight, they have, without resting from their arduous labours, at once set about devising methods calculated to break and destroy the cruel power which these innumerable hosts have so long exercised, almost undisputedly, over defenceless mankind, and the animal kingdom in general.

There were, indeed, weighty reasons for pushing the inquiry in this direction, for the abundant experience of zymotic disease in the past gave strong indications of the kind of way in which possibly these diseases might some day be brought into subjection, and their destroying power curtailed. It has always been a familiar observation, that one attack of a zymotic disease generally protects the individual from a second attack of the same disease, for although there are some persons unfortunate enough to suffer twice or even three times from measles or scarlet fever, still we all know that in by far the greater number of cases persons suffer but once from these and most other zymotic diseases. It is also a fact, with which physicians have long been familiar, that the intensity of these diseases is subject to great variations, not only in different persons, but also at different times and in different places. Thus sometimes scarlet fever or diphtheria will appear in a place as very serious diseases, with a large number of bad cases and many deaths, whilst at another time or in another place they may come in a much milder form, with few deaths and few serious cases. These remarkable phenomena early attracted great attention in the case of small-pox, in which the further extraordinary observation was made, that if the disease had been accidentally communicated by direct inoculation from one person to another, especially in childhood, the attack was generally a lighter one than usual, the person being protected against further attacks in the future. This led to the custom of regularly inoculating with small-pox, which was much practised in the East, and was introduced into this country in the last century, and much resorted to until the epoch-making discovery of Jenner, in 1799, nearly 100 years ago, that persons might be similarly protected by being inoculated with *cow-pox*, a disease not unfrequently affecting the cow and the horse, but not naturally occurring in man. Inoculation with this disease, or *vaccination* as it is generally termed, had the advantage over the inoculation with small-pox, that it gave rise to an even still milder disease, and less dangerous than the mild form of small-pox which generally results from inoculation. This great discovery, made so many years ago, and which has so greatly mitigated the ravages of small-pox, is all but universally regarded as one of the greatest triumphs of Medicine, and indeed the foundation-stone of that department of Medicine which we now commonly call Preventive Medicine, and for which the immediate future extends hopes of still more majestic development than the past. This discovery of Jenner's, then, remained an isolated achievement, until the careful study of micro-organisms and their effects, during the past fifteen years, threw fresh light upon these wonderful and hitherto puzzling phenomena.

The new ground in this direction was first broken by Pasteur, in the course of his investigation of a very fatal zymotic disease which affects poultry-yards, and which is known as Chicken-Cholera.

Pasteur found that if the artificial cultures of this bacillus were preserved for a period of nine or ten months, they produced on inoculation into fowls only a trifling instead of the fatal malady, yet the birds were thereby permanently protected from subsequently contracting the disease. Moreover, on making fresh artificial cultures from this attenuated virus, they also were found to be only capable of setting up the milder form of the disease. Thus in this simple manner Pasteur succeeded in artifi-

Fig. 41.—Bacillus of Chicken Cholera.

cially cultivating a race of Chicken-cholera bacilli not only devoid of fatal properties, but able also to protect from subsequent inroads of the malignant bacilli. This discovery made in 1877 was a most material advance in the study of the great question of Preventive Inoculation, and although practically affecting only the welfare of the humble barn-door fowl, and the pocket of the poultry-farmer, was still an earnest of the triumphs over zymotic disease that were at hand.

The next disease to receive attention from this point of view was *Anthrax*, or *Woolsorters' disease*, which we have seen is so fatal, both to man and many of the lower animals, especially horned cattle, sheep, rabbits, guinea-pigs, and above all white mice.

The methods adopted for obtaining protection or immunity from Anthrax have been of the most varied and instructive character, and it will be well worth our while to investigate them more closely. In carrying on his studies upon Anthrax, Pasteur found that the virulence of the bacilli was greatly impaired by a temperature only very slightly in excess of blood-heat, and that if they were kept in artificial cultures at a temperature of 42—43° C. for a period of 43 days, they were so much weakened as to be no longer fatal even to the most sensitive animals. Moreover, on starting fresh cultures from these weakened ones, the new cultures were found to preserve the character of the old ones, and were from the first of the same non-fatal kind; animals inoculated with them suffer scarcely any indisposition, but are notwithstanding perfectly protected from Anthrax.

Pasteur did not delay in making this brilliant laboratory achievement available for practical application, and for nearly ten years past this attenuated virus has been prepared on a large scale under M. Pasteur's superintendence for distribution throughout the world. Pasteur's first great experiment with this attenuated virus outside the laboratory is particularly noteworthy. On the 5*th of May*, 1881, there were brought to him twenty-four sheep, one goat, and six cows, all of them animals eminently susceptible to this disease. They were all inoculated on this day with Pasteur's attenuated virus, and twelve days later they were again inoculated with a rather less attenuated virus.

On May the 31st, all these inoculated animals, as well as twenty-four sheep, one goat, and four cattle not previously inoculated, received severally an injection with virulent anthrax. On June the 2nd, or three days later, twenty-one of the sheep and the goat which had not been inoculated were dead, two other sheep were dying, and the last one was also attacked later on in the day, *whilst not one of the previously inoculated animals was affected.* It is difficult for any one not engaged in scientific pursuits to fully realize how the triumph of that moment must have rewarded the years of patient and religious labour of the Seeker after Truth. The extent to which this protective treatment has been taken advantage of may be gathered from the following figures recently communicated by M. Pasteur to Sir Joseph Lister—

Two and a half million sheep, 320,000 horned cattle, 2,861 horses, have been inoculated from the Institut Pasteur, whilst in 1888-9 material was also sent to India for the inoculation of 1000 elephants.

Within the last few years great attention has been bestowed on the chemical substances which these disease-producing organisms generate, and numerous investigators have succeeded in calling forth many of the symptoms of a disease by injecting the products of these organisms from which the organisms themselves had been carefully removed. But not only has it been found that these products free from organisms are capable of inducing the symptoms of a disease, but the animal so experimented on is often thereby protected from the disease itself. This method of securing immunity by means of bacterial poisons has been most beautifully worked out for anthrax by Mr. Hankin of Cambridge. Mr. Hankin has succeeded in isolating from anthrax-cultures a substance which has many points of similarity to snake-poison; he calls it the *Toxalbumose of Anthrax*, and he finds that this substance is so poisonous that if he injects into a rabbit a dose of not more than $\frac{1}{5000000}$ of its body-weight the animal is killed, whilst if he takes only half that amount, or $\frac{1}{10000000}$ of its body-weight, the animal does not die, but on the contrary is thereby protected against anthrax. Indeed, by carefully proportioning the dose of this toxalbumose, Hankin has actually succeeded in protecting white mice, which are the most susceptible of all animals to anthrax.

The most obvious explanation of these remarkable results would appear to be that the introduction of a suitable quantity of this poison into the animal system accustoms the animal to the action of the poison, and prepares the animal to successfully withstand the larger quantities of this poison which are generated within it when the virulent anthrax bacilli are subsequently inoculated. We are all of us familiar with the fact that the system can by practice be made to tolerate considerable doses of powerful poisons ; thus the habitual smoker can endure without inconvenience doses of nicotine, the poisonous principle of the tobacco-leaf, which would make a sorry sight of the inexperienced novice to the fragrant weed! The curious point of difference between the two phenomena is, that in the case of anthrax the animal becomes habituated to the poison by a single injection, whilst the tolerance of tobacco is only acquired after a prolonged apprenticeship ; it is in fact as though the first cigarette should prepare a man to forthwith venture upon a full-flavoured cigar, a proceeding the effects of which it is unnecessary to say would stamp themselves upon the memory for the remainder of his life.

Something similar to Hankin's results had been previously observed in the case of snake-poison, for it had been found that by injecting very minute doses of the essential principle of this poison into pigeons, they acquire the power of withstanding no less than seven times the ordinary deadly dose of snake-poison, even if administered three months afterwards.

But of all communicable diseases there is probably none which inspires such absolute terror and consternation as Hydrophobia or Rabies, and although the disease is fortunately so rare that comparatively few persons have actually seen a human being suffering from it, still the terrible nature of the disease is so well known that the dread of it is shared by all.

It was doubtless the appalling character of this disease and the helplessness of physicians to cope with its attacks that first led Pasteur to turn his great mind to its investigation now eleven years ago. Peculiar difficulties have surrounded the study of this disease, for in spite of the most careful and long-continued attention that has been given to it by some of the best observers of the day, all have hitherto failed in discovering the exciting cause, although of course no doubt can exist, from the manner in which it is communicated, that it must be due to an organized poison, more or less similar in character to those which we have already been considering. But although even Pasteur's eagle eye was unable to discover the exciting cause of hydrophobia, he was, by the scientific use of the imagination, and by ingenious experiments, successful not only in devising a method for protecting the individual against the virus of hydrophobia, but actually in warding off the attack of this disease by means of a treatment which is only put into operation after the infection has taken place. This marvellous discovery, which is assuredly one of the grandest achievements of scientific intuition and deductive acumen, it is which has made M. Pasteur's laboratory in Paris the Mecca for anxious pilgrims coming from the east and the west, from the north and the south, speaking a Babel of languages, some clothed in rags and some in soft raiment, but all possessing one thing at least in common—the gnawing care of having been bitten by a mad dog, cat, jackal, or rabid animal of one kind or another. Such, indeed, was the gathering which in 1886 I myself witnessed in the small building hastily extemporized for M. Pasteur's purposes, and which has since been replaced by the palatial Institute erected by the joint efforts of a grateful community and of a prudent Republican Government to afford a wider arena for the commanding genius of Pasteur.

We will now endeavour to make ourselves acquainted with the leading features of this preventive treatment of Rabies, the report of which has reached to the uttermost parts of the earth.

The starting-point of Pasteur's experiments on Hydrophobia was a child suffering from this disease, as the result of being bitten by a mad dog. He took some of this child's saliva and inoculated it under the skin of a rabbit. The rabbit was dead in two days; and on inoculating the blood of this first rabbit into a second, the latter was also found dead in two days. This rapid course of the disease is so unusual in hydrophobia that it appeared very doubtful as to whether he was really dealing with this disease, and not some other form of infection.

In subsequent experiments Pasteur invariably adopted the plan of inoculating with the brain of the affected animal into the brain of a healthy one, as in this manner the disease was produced with most certainty, and of the most uniform character, as long as the same kind of animal was employed. Pasteur was now guided by his experience gained in dealing with anthrax, and in which he had observed that the virulence of the disease would be modified by passing from one species of animal to another. Inoculating from hydrophobic dogs into rabbits, he found that the virulence of the disease was greatly intensified, death following much more rapidly after inoculation than in the rabies of dogs. On the other hand, by inoculating from dogs into monkeys, he found that the virulence of the disease became diminished, and after being transmitted several times from monkey to monkey, the malady ceases to be fatal. It was thus possible to obtain virus in all degrees of malignity—*virus of great strength from the rabbit, of medium strength* *from the dog, and of low strength from the ape.* Pasteur now endeavoured to accustom an animal to the rabies-poison by treating it successively with poison of increasing strength; this was done by preparing a series of rabbits which were to yield the poison of different degrees of virulence. The first rabbit was inoculated with the weakened virus from a monkey, from this rabbit the disease was communicated to a second, from that to a third, and so on in series, the intensity of the virus increasing as it passed from one rabbit to another.

With this graduated scale of poisons he now attempted the protection of dogs. Twenty dogs were each successively inoculated with the graduated rabbit-poison, beginning with the weakest, and giving a stronger poison at each successive inoculation. After these twenty dogs had undergone this treatment, they were each exposed to infection with virulent hydrophobia, the result being that three-fourths were found to resist the infection, which was so eminently satisfactory that it stimulated Pasteur to further perfect the process. He now found that the graduated scale of hydrophobia-poison could be obtained in a more simple manner than that which I have described, for he observed that by keeping the spinal marrow of a rabbit which had died of rabies in a dry atmosphere its virulence became weakened from day to day, and after about fifteen days the virulence had almost completely disappeared. In this way a graduated series of poisons can be obtained by having a series of spinal cords, the strongest being one day old and the weakest fifteen days old. With such a series of poisons, obtained in this extremely simple manner, Pasteur now commenced the treatment of fifty dogs. Each dog was submitted to a treatment extending over ten days, during which period it received a number of successive inoculations with the rabbit-marrows of different ages, beginning with the oldest and therefore weakest virus, and ending with the youngest and strongest virus. On subsequently exposing these fifty dogs to infection with virulent hydrophobia, they were found to be all protected, and this protection proved to be permanent for at least two years.

With such results obtained in the laboratory, Pasteur now felt justified in extending the treatment to human beings. The first case treated was that of a child bitten on July 4th, 1885, since which a long string of patients have passed through the process, both at the Institut Pasteur and the other places where arrangements have been made for bestowing upon suffering humanity the fruits of this latest departure of scientific research. Such institutions have been established in Russia, Hungary, Italy, Sicily, Brazil, Mexico, Turkey, the United States, and Roumania, whilst in Great Britain, to our unutterable disgrace, we are in this respect behind the unspeakable Turk, and the semi-barbarous subjects of the Czar.

At the Institut Pasteur the following numbers have been treated—

Year.	Number Treated.	Number Died.	Percentage.
1886	2,671	25	·94
1887	1,770	13	·73
1888	1,622	9	·55
1889	1,830	6	·33
1890	1,540	5	·32

The mortality amongst persons bitten by rabid dogs and not submitted to treatment may be taken at 15—20 *per cent.*

The chance of escape is mainly dependent upon two circumstances, of which the *first* is the time which has elapsed between the infliction of the wound and the commencement of the treatment; if the patient die within fifteen days after the completion of the inoculations, it is considered that the case was from the first a hopeless one, as it shows that the disease had already commenced its activity during the treatment, and such cases are not recorded in these statistics; and *secondly*, the gravity and position of the wound are of much importance in determining the chance of successful treatment. Thus in the case of the patients treated in 1890, the mortality for

Bites on the Head was	·85 per cent.
,, ,, Hands was	·45 ,,
,, ,, Limbs and body was	·00 ,,

Again, bites by mad wolves are far more dangerous than those by mad dogs, as the virulence of the disease is greater in the wolf than in the dog.

In the following table I have recorded the most recent method of treatment which has been pursued at the Russian Military Station at Tiflis during the year 1890. The patient is subjected to two distinct series of inoculations; the first extending over nine days, stronger and stronger virus being employed from day to day; whilst in the second series, which extends over ten further days, a return is made to weak virus, which is then again strengthened from day to day as indicated in the table—

I. Series.

1	Day	12	Days'	Marrow
1	,,	10	,,	,,
1	,,	8	,,	,,
2	,,	6	,,	,,
2	,,	5	,,	,,
2	,,	4	,,	,,
3	,,	4	,,	,,
3	,,	3	,,	,,
3	,,	2	,,	,,
4	,,	2	,,	,,
4	,,	1	,,	,,
5	,,	1	,,	,,
6	,,	5	,,	,,
7	,,	4	,,	,,
7	,,	3	,,	,,
8	,,	2	,,	,,
8	,,	1	,,	,,
9	,,	1	,,	,,

II. Series.

10	Day	8	Days'	Marrow
10	,,	7	,,	,,
11	,,	6	,,	,,
11	,,	5	,,	,,
12	,,	5	,,	,,
12	,,	4	,,	,,
13	,,	4	,,	,,
13	,,	3	,,	,,
14	,,	3	,,	,,
14	,,	2	,,	,,
15	,,	2	,,	,,
16	,,	1	,,	,,
17	,,	5	,,	,,
17	,,	4	,,	,,
18	,,	3	,,	,,
18	,,	2	,,	,,
19	,,	1	,,	,,

It is only natural that scientific thinkers should have endeavoured to systematize the vast mass of accumulated facts concerning the relationship between the animal system and these various micro-organisms which have the power of living parasitically upon it, and causing it so much mischief. Numerous theories have indeed been propounded to account on the one hand for the injuries which these organisms are capable of doing the body, and on the other hand of the power which the body possesses of warding off their attacks. The construction of a single theory harmonizing with all the observed facts in this matter is attended with the greatest difficulties, nor have these difficulties been yet overcome, as we are still without any working hypothesis which gives a satisfactory explanation of *all* the phenomena to which I have referred.

This would obviously not be a suitable place to balance the arguments for and against the various theories which have been suggested in explanation of these matters ; but there is one theory, which on account of its extreme ingenuity and the large amount of experimental evidence which has been marshalled in its support by its originator, I cannot refrain from referring to. In studying these communicable diseases it becomes obvious even to a superficial observer that a species of contest or warfare takes place between the micro-organisms of these diseases and the body of the animal they infest. In some cases the micro-organisms, and in other cases the body is victorious; in some again the battle is long, in others it is short. In the case of animals highly susceptible to a disease, the victory almost invariably lies with the microbes, whilst in the case of those which enjoy a natural immunity, or in which immunity has been artificially conferred by means of one of the methods of protective treatment which I have mentioned, the victory is secured by the body.

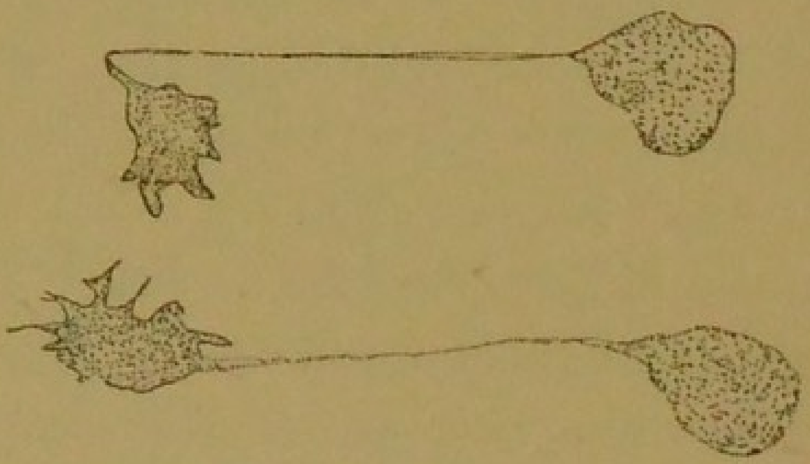

Fig. 42.—"A common or pale colourless corpuscle is shown after it has separated into two lumps, connected by a thin bridge. The lower of the two figures is a later stage of movement of the one above."—*Atlas of Histology*, by E. Klein and E. Noble Smith.

M. Metchnikoff, an illustrious Russian bacteriologist, conceives of this struggle on behalf of the body being carried on by certain small living cells or corpuscles which are present in the blood and some of the other fluids which bathe the tissues of the living body. The cells to which is entrusted this warfare include the ordinary colourless corpuscles and other cells, to all of which the collective name of *Leucocytes* has been given (see Fig. 42). These leucocytes are endowed with an individuality of their own, having the power of independent locomotion, which they accomplish by what is known as amœboid movements.

In the accompanying figure such a leucocyte is represented executing one of these remarkable movements.

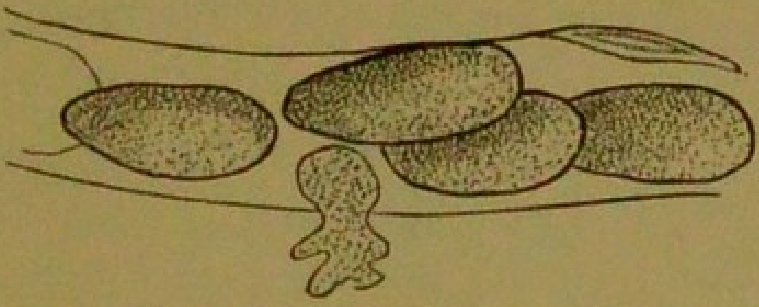

Fig. 43.—Leucocyte commencing to traverse the wall of a capillary. (*After Metchnikoff.*)

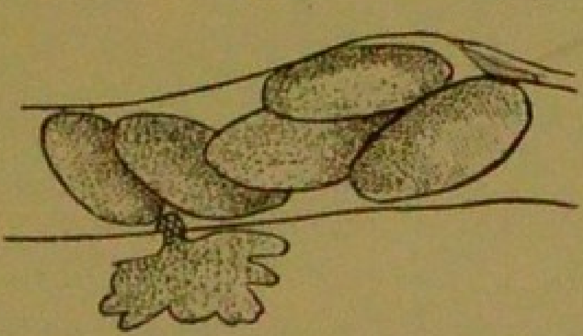

Fig. 44.—The same leucocyte as in Fig. 43, at a later stage. (*After Metchnikoff.*)

In the first of these figures a leucocyte of irregular contour is represented as commencing to traverse the wall of a blood-vessel, the latter being filled with oval blood-corpuscles. The second figure represents the same leucocyte after a short interval of time, thus exhibiting the amœboid movements by means of which these bodies propel themselves.

It has long been known that if fine solid material is injected into the blood, these solid particles become surrounded by such leucocytes, and if of a digestible nature they become assimilated by the leucocyte, but if unsuited for digestion these particles are ejected as shown in the next figure.

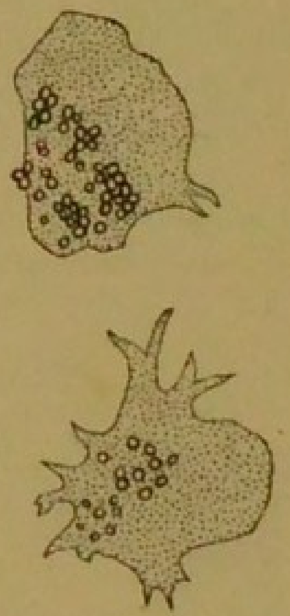

Fig. 45.—Leucocytes containing particles of vermilion. The lower figure represents the same leucocyte after it has got rid of some of its load. (*After Klein and Noble Smith.*)

There is, thus, no question that these leucocytes act as the scavengers of the blood, but Metchnikoff conceives of them as not only performing these menial duties, but also endowed with a martial spirit which is called into activity when the body is threatened with an invasion by the bacterial hordes. As soon as these enemies of the body effect an entrance at any point, the leucocytes, according to M. Metchnikoff, muster in large numbers, and close in deadly contest with the invaders. In this struggle the leucocytes avail themselves of their voracious appetite, and enclose within their capacious bodies great numbers of their Liliputian foes, whilst the latter employ as their engines of war the deadly poisons which we have seen they are capable of manufacturing with such rapidity and skill.

Nor must it be supposed that this picture is a mere romantic flight of fancy on the part of the imaginative Russian savant; on the contrary, his theory is based on the most careful observations and experiments made by himself and others.

In elaborating his theory of Phagocytosis, as this particular behaviour of certain cells present in the tissues and blood-vessels of the body is called, Metchnikoff has not restricted his study to their behaviour in the higher vertebrata, including man himself, but has gone back to the most rudimentary forms of life in the animal kingdom, commencing with the unicellular organisms, or protozoa in which the nervous and vascular systems are entirely absent.

In thus commencing with the simplest and working up to the most complex organisms, tracing the development of particular pathological processes from their beginning to their consummation, Metchnikoff has simply followed the path dictated by the principles of evolution, and has with marvellous skill and ingenuity pursued and marked out the intricate progress of these phenomena from species to species. Thus in his own words:[1] "Il n'est point nécessaire de fournir de preuves spéciales pour affirmer que la maladie et les processus pathologiques ont leur évolution comme l'homme et les animaux supérieurs eux-mêmes. Chez tous les organismes, à partir des êtres les plus inférieurs, nous trouvons déja des maladies infectieuses produites par des parasites appartenant

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[1] "Leçons sur la pathologie comparée de l'Inflammation," par Élie Metchnikoff. Paris, 1892.

à des groupes différents. Il est donc tout naturel de supposer que ce parasitisme occasionne une série déterminée de troubles dans l'organisme infecté et provoque aussi des phénomènes réactionnels de la part de ce dernier."

Now although from what has been said these leucocytes would appear to have a voracious appetite, inasmuch as they will engulph such unremunerative materials as particles of vermilion, yet in reality their tastes are by no means so catholic as might be imagined. They are, on the contrary, endowed with a highly discriminating palate, and this discrimination not only renders them capable of exercising a most refined selection in what they will engulph, but determines their movements also. Thus if the mesentery of a frog be moistened with a solution of quinine, the leucocytes remain quietly in the interior of the blood-vessels, in spite of the dilated condition of their walls affording them the most favourable opportunity for migration. It was at first supposed that this inaction on the part of the leucocytes was due to the quinine having paralyzed them, inasmuch as it acts deleteriously on the protoplasm of the tissues, but on repeating the experiment whilst confirming their negative behaviour under these circumstances, the surprising fact was revealed that on removing such leucocytes from the blood-vessels, their inherent motility had been in no way impaired, their amœboid movements being as characteristic as ever. Now this showed in the most striking manner that their previous immobility was due to a deliberate determination on the part of these leucocytes to have nothing whatever to do with the particles of quinine, and also that this decision had been arrived at in consequence of information of some sort or other which had penetrated to them when shut up within the walls of the blood-vessels. This gift of apparent discrimination and marked individuality of action which leads them to be attracted by some substances and repelled by others is now generally known as *Chimiotaxis.*

In the case of micro-organisms this chimiotaxis is exhibited in a most remarkable manner. Some varieties are absorbed by the leucocytes and others are refused, and refused moreover in some cases where their destruction by the leucocytes is of the most vital importance to the organism to which the leucocyte belongs. This seemingly unaccountable behaviour of the leucocyte has been attributed to the chemical products elaborated by different bacteria within the animal on which they are parasitic, the substances produced by some causing attraction, those produced by others causing repulsion of the leucocytes.

In many of the most virulent and fatal types of disease, such as chicken-cholera, the leucocytes stand aside altogether, and refuse to have anything to do with the microbes. This fact may possibly account for the great mortality which we have seen occurs amongst fowls infected with this chicken-cholera, and here in the absence of the necessary powers to throw off the disease in the animal itself, man has had to interpose with artificial expedients, enabling the animal to successfully combat with its most unwelcome parasite. But this negative behaviour of the leucocytes in such cases in which their action would be of primary importance to the animal affected, has naturally afforded a favourable opportunity for the opponents of M. Metchnikoff to attack his leucocytic-theory.

But the ingenious Russian is not easily defeated, and he again shelters himself under the mantle of evolution. He urges that just because the development of the powers of the phagocytes takes place in accordance with the laws of natural selection, and not in pursuance of any one predetermined path, we find instances where, in consequence either of their functional activity being suspended or being prejudicially exercised, the well-being of the organism is not only gravely menaced, but death itself frequently ensues. This all takes place, says M. Metchnikoff, because the functions of the phagocytes are not yet perfected, because their development is still going on, and it is this gradual evolution of their powers which has rendered possible the intervention of human skill in assisting the organism to perform what it is not yet capable of accomplishing by itself.

This theory of Phagocytosis is so interesting and important, and opens up such a wide field for speculation and research, that I must be pardoned for having dealt with it in more detail than perhaps the compass of this little work would warrant. In conclusion, I will endeavour to show in the following figures the nature of the struggle which is supposed to take place between certain bacteria and the leucocytes.

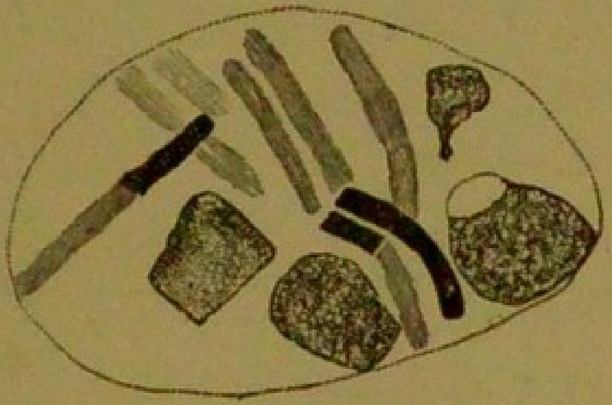

Fig. 46.—Leucocyte of White Rat, enclosing Anthrax bacilli. (*After Metchnikoff.*)

In this figure (Fig. 46) a large oval cell or leucocyte is represented as having engulphed a number of rod-like bodies, the bacilli of anthrax, which are undergoing gradual degeneration and decay within; thus those bacilli which are still possessed of

Fig. 47.—Leucocyte of Monkey, enclosing spirilla of recurrent fever. (*After Soudakewitsch.*)

vitality are of their normal size and sharply defined, whilst those which have suffered partial resolution are swollen up, and but faintly marked.

In Fig. 47 the large irregular cell or leucocyte is represented enclosing a number of the coiled spirilla of recurrent fever, whilst in the following figure (Fig. 48) the mortification and decay of one of these spirilla is exhibited through its becoming uncoiled and disjointed.

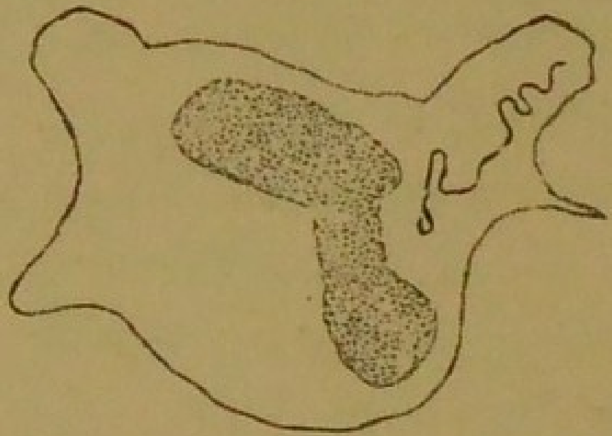

Fig. 48.—Leucocyte of Monkey, containing spirillum of recurrent fever, showing the disintegration of the latter. (*After Soudakewitsch.*)

In this contest between the leucocytes and the bacteria, according to Metchnikoff, if the leucocytes are defeated, the animal falls a victim to the disease, whilst if the leucocytes are victorious and the bacteria exterminated, the animal recovers, and the severity of the disease will depend upon the severity of the struggle between the combatants.

Moreover, if the leucocytes have been once successful in such a contest with a particular species of bacteria, they will on a future occasion secure the victory over the same species with far greater ease; and on this account one attack of an infectious disease commonly protects, as we have seen, either temporarily or permanently, against a second attack of the same disease. Also, if the bacteria are introduced in a weakened or attenuated condition, the leucocytes naturally have an easy task in subduing them, yet by the experience gained in this trivial contest they are often able to successfully oppose the inroads of the most virulent forms of the same species. This is in fact the manner in which the upholders of Metchnikoff's theory account for the phenomenon of immunity acquired by protective inoculation. Indeed it has been shown by actual experiment that if the bacteria are defended from the attacks of the leucocytes, they can actually multiply abundantly in the blood of the immune animal. Thus, if the spores of virulent anthrax are enclosed in a little paper packet, and this is introduced under the skin of a rabbit which has been rendered immune towards anthrax, the spores germinate inside the paper envelope, and multiply abundantly therein, but any of the anthrax bacilli getting outside the envelope immediately fall into the clutches of the leucocytes and are destroyed.

But we have seen that immunity may also be secured by protective inoculation, not with weakened or attenuated bacteria, but with the chemical products elaborated by the particular bacteria. This method of obtaining immunity is supposed to depend upon the leucocytes becoming thereby accustomed to the particular bacterial poisons in small doses, and thus being enabled to cope more easily with the generators of these same poisons when they are introduced in person into the body of the animal.

It is, however, very difficult to harmonize with this theory some exceedingly remarkable and interesting observations recently made by Kitasato, to whom reference has already been made. Kitasato in the course of his investigations on the phenomena connected with tetanus or lockjaw, found that it is possible to secure immunity from this disease by injecting into rabbits a small quantity of a chemical substance known as Trichloride of Iodine. This discovery is remarkable enough in itself, but still more wonderful is that which followed, viz. that on injecting only a few drops of the blood of this protected rabbit into a mouse, the latter also became protected against tetanus. And further, he found that mice already infected with tetanus, and in which the tetanic symptoms had already commenced in several of the extremities, could still be rescued by having recourse to an injection with the blood of rabbits which had been protected against tetanus with the trichloride of iodine.

This remarkable action appears to be due to the extraordinary power possessed by this protected rabbits' blood of destroying the particular poison generated by the tetanus organism, and this destructive power can actually be demonstrated outside the animal body altogether in the most conclusive manner.

Thus Kitasato took an artificial culture of the tetanus bacilli, which had been growing for ten days. This culture-liquid he passed through a filter to remove the bacilli, and the clear liquid so obtained is of the most intensely poisonous character, for ($\frac{1}{20000}$ c.c. or) $\frac{1}{1000}$ of a drop is sufficient to kill a mouse in 4—6 days. (One c.c. or) twenty drops of this poisonous liquid were mixed with 5 c.c. of the blood-serum of a protected rabbit, and after twenty-four hours a quantity of this mixture (containing 300 times the fatal dose) was inoculated into each of four mice, but not one of them was affected, thus clearly showing that the tetanic poison had been destroyed by the twenty-four hours' contact with the rabbit's blood-serum.

These results are so striking that they require but little comment; it is obvious, however, that they reveal to us the possibility of combating disease with entirely new agents, and who can say to what achievements the path thus inaugurated by Kitasato may not lead in the future?

The youthful science of Bacteriology is indeed full of surprises, and every year bears witness to the astounding strides with which it grows, whilst day by day it is being more widely recognized of what stupendous importance to man is the message which it has to deliver.

During the earlier childhood and adolescence of this new science, it was generally believed that this message was of a gloomy character, and one which it would be better to leave untold, for to the public it seemed as though it had nothing but death and destruction to reveal as lurking where danger was hitherto unsuspected. For many years Bacteriologists had apparently nothing to announce but the discovery of new and subtle enemies to mankind, and to produce poisons possessing such a degree of malignity that beside them the venom of snakes and the most potent drugs of the apothecary appeared as comparatively harmless or even friendly. In reality, however, these deadly foes and poisons have always existed before, and have wrought their lethal work in the dark until exposed and branded by men of science, who after years of patient labour are now teaching the world how these foes may be vanquished, and how these old but until recently undiscovered poisons may be counteracted and rendered innocuous by the administration of New Antidotes.

Richard Clay & Sons, Limited,
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NUMBER OF COLONIES OBTAINED FROM 1 CUBIC CENTIMETRE OF WATER BY GELATINE-PLATE CULTIVATION.

TABLE I. 1886.

Name of Supply.	Janry.	Febry.	March.	April.	May.	June.	July.	August.	Septr.	Octr.	Novr.	Decr.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Average for Year.
													Reduction per cent.												
THAMES.																									
Thames Water, unfiltered	45,000	15,800	11,415	12,250	4,800	8,300	3,000	6,100	8,400	8,600	56,000	63,000	95·6	98·6	95·3	99·1	98·8	98·9	95·6	94·0	99·4	99·4	98·8	98·3	97·6
Chelsea	159	305	299	94	59	60	59	303	87	34	65	222													
West Middlesex	180	80	175	47	19	145	45	25	27	22	47	2,000													
Southwark	2,270	284	1,562	77	29	94	380	60	49	61	321	1,100													
Grand Junction	4,894	208	379	115	51	17	14	12	17	77	80	1,700													
Lambeth	2,587	265	287	209	136	129	155	1,415	59	45	108	305													
LEE.																									
Lee Water, unfiltered	39,300	20,600	9,025	7,300	2,950	4,700	5,400	4,300	3,700	6,400	12,700	121,000	99·4	98·8	94·1	96·3	95·2	90·5	97·5	94·3	95·5	98·5	98·0	99·8	96·5
New River	363	74	95	60	22	53	46	55	17	10	32	400													
East London	224	252	533	269	143	445	134	243	165	97	248	280													
DEEP WELLS.																									
Bath Well	—	—	—	—	—	—	—	—	—	—	12	10													
Garden Well	—	—	—	—	—	—	—	—	—	—	—	—													
New Well	— —	5* —	44* —	7* —	8* —	4* —	12* —	9* —	5* —	3* 160*	—	11													
Supply	43	149	38	47	101	39	48	13	25	283 405	196	66													

* In all cases marked with an asterisk the name of the particular well was not mentioned.

TABLE II. 1887.

Name of Supply.	Janry.	Febry.	March.	April.	May.	June.	July.	August.	Septr.	Octr.	Novr.	Decr.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Average for Year.
													Reduction per cent.												
THAMES.																									
Thames Water, unfiltered	30,800	6,700	30,900	52,100	2,100	2,200	2,500	7,200	16,700	6,700	81,000	19,000	87·6	96·0	98·1	99·7	97·7	89·7	96·7	97·2	99·6	98·8	99·6	99·4	96·7
Chelsea	5,300	81	171	55	49	190	106	44	73	64	187	86													
West Middlesex	258	27	96	110	32	123	40	87	82	28	43	113													
Southwark	4,900	428	1,325	360	61	196	119	70	84	130	152	133													
Grand Junction	7,500	612	443	109	48	103	35	78	15	80	55	80													
Lambeth	1,200	188	884	103	53	521	108	733	85	96	1,120	198													
LEE.																									
Lee Water, unfiltered	37,700	7,900	24,000	1,330	2,200	12,200	12,300	5,300	9,200	7,600	27,000	11,000	82·2	98·7	99·2	90·5	95·2	90·2	98·4	98·0	98·2	98·1	99·3	95·9	95·3
New River	508	72	133	38	16	31	33	15	23	25	41	39													
East London	6,700	100	182	127	105	1,200	194	104	169	148	190	456													
DEEP WELLS.																									
Bath Well	9	19	80	26	27	12	14	5	5	7	3	6													
Garden Well	48	20	4	4	—	24	18	—	8	—	5	12													
New Well	12	10	5	12	20	14	8	59	27	30	65	67													
Supply	82	75	140	163	50	26	44	116	115	357	40	68													

TABLE III. 1888.

Name of Supply.	Janry.	Febry.	March.	April.	May.	June.	July.	August.	Septr.	Octr.	Novr.	Decr.	January.	February.	March.	April.	May.	June.	July.	August.	September.	October.	November.	December.	Average for Year.
													Reduction per cent.												
THAMES.																									
Thames Water, unfiltered	92,000	40,000	66,000	13,000	1,900	3,500	1,070	3,000	1,740	1,130	11,700	10,600	99·9	98·9	99·4	99·3	96·8	98·1	96·8	99·3	96·8	97·8	99·3	98·9	98·4
Chelsea	127	152	54	38	43	63	37	32	36	14	82	71													
West Middlesex	60	146	408	158	71	56	27	11	26	33	31	16													
Southwark	177	766	742	47	47	24	35	27	106	35	167	136													
Grand Junction	90	349	617	56	77	40	15	4	20	16	25	208													
Lambeth	189	820	321	157	64	140	55	33	92	27	126	151													
LEE.																									
Lee Water, unfiltered	31,000	26,000	63,000	84,000	1,124	7,000	2,190	2,000	1,670	2,310	57,500	4,400	93·4	97·0	99·4	99·8	81·4	96·2	88·4	97·2	96·2	97·3	99·9	96·8	95·3
New River	27	90	169	77	37	60	11	13	—	15	70	91													
East London	2,038	780	359	193	209	266	253	57	64	63	49	141													
DEEP WELLS.																									
Bath	6	47	6	33	7	17	8	—	8	4	34	—													
Garden	5	19	8	4	27	71	5	—	10	9	18	—													
New	12	4	5	7	8	20	4	3	—	96	19	—													
Supply	55	81	15	69	139	219	32	42	52	55	54	63													

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