(16)

The Origin *and* Distribution *of* Microzymes (Bacteria) *in* Water, *and the* Circumstances *which determine their* Existence *in the* Tissues *and* Liquids *of the* Living Body. By Dr. Burdon-Sanderson, F.R.S.

(*Reprinted, by permission, from the author's second "Report of Researches concerning the Intimate Pathology of Contagion," in the Appendix to the '13th Report of the Medical Officer of the Privy Council.'*)

In my previous report on the intimate pathology of contagion, microzymes were defined as living particles which in their earliest state are spheroids, and do not exceed $\frac{1}{20,000}$ of an inch in diameter, but subsequently elongate into rods. As regards the conditions of their development, their existence was said to be associated with the commencement of putrefactive decomposition of nitrogenous compounds. The question of their origin and destiny was left unanswered. It was left undecided whether on the one hand "they constitute a race of more or less similar individuals, each of which springs from and reproduces its like," or, on the other, are "germs in which a specific form is wrapped up," capable of developing to the higher organisms from which they spring.

It is to this question principally that the experiments we have now to bring before the reader relate. Our purpose is to examine into the origin, growth, and development of microzymes, to investigate the conditions which are fatal or favourable to their existence in the liquid and gaseous fluids by which we are surrounded, in the hope that by doing so we may be enabled to approach one degree nearer to an understanding of their influence on the processes which go on in the living body.

In dealing with the question of origin, we again encounter the more general question of what is called "spontaneous generation." I have no intention, however, of entering upon it. I shall be able to prove in the most decisive manner that as regards the animal liquids and tissues, and the liquids which will be used as tests for the presence of microzyme germs, no spontaneous evolution of any organic form ever takes places; but it will be quite unnecessary either to deny or to assert its possibility under other and different conditions.

Before proceeding to state the results of our experiments, a more complete account must be given of microzymes, and something must be said as to the views entertained by naturalists of their nature, origin, and relation to other organic forms. The methods of investigation which have been employed must also be explained.

Bacteria or microzymes are placed by most naturalists in the animal kingdom, and have a position assigned to them next to the monads. Hallier, on the other hand, believing that they originate by the cleavage and multiplication of nuclei in the cells of fungi, and that they develop to the same forms from which they spring, regards them as plants. Their claim to be considered animals is founded partly on their motions, partly on the fact that their chemical reaction on air, when alive, resembles rather the respiration of animals than that which is associated with vegetation. The question is of importance only in so far as it involves that of origin and development. If it can be shown that they neither spring from higher forms nor grow to them, the discussion of their animal or plant nature may be left to those interested in verbal definitions.

Microzymes grow either in liquids or moist air. In liquids they present different appearances, as they are observed in the depth or on the surface. In the former case they show no tendency to assume any special arrangement to each other if they are motionless; nor if they are active are their motions governed by any mutual relation. At the surface of the liquid, on the other hand, although the individual bacteria show no definite arrangement when they first appear, they soon place themselves in such a manner as to form a membrane, the beginning of the bacterium scum, to which we shall have frequent occasion to refer. In this membrane, when it first appears, each rod stands vertically, one end forming part of the free surface, the other part of the deep surface of the membrane. The rods adhere together by their sides after the manner of the elements of columnar epithelium, but there is, I think, strong reason to believe that this adhesion is not direct, *i. e.* that they are not in actual contact, but glued together by a viscous intermediary substance. Consequently on this arrangement the "scum," when first formed, presents under the microscope the aspect of an evenly dotted surface, the distance between each dot and its neighbour corresponding approximately to the diameter of a rod. This appearance, indeed, is so deceptive, that for a long time I supposed, as others have done, that the constituent particles were round; nor was it until it was discovered that the mem-

brane could be resolved by mechanical means into rods that I understood the real nature of the membrane. As the structure (if one may call it so) becomes thick enough to form a visible scum, the arrangement of the particles can no longer be made out, for it is not possible to subject it to examination without dislocating it to such a degree as to render their relative positions indistinguishable.

When common microzymes grow on moist surfaces, they with their intervening jelly sometimes form viscous masses of sufficient size to be cognoscible by the unaided senses, these consisting of a material similar to that of the "scum" which forms on the surface of liquids in which microzymes are growing. This fact is expressed by the term *Zooglæa* applied to such masses or colonies of microzymes by Cohn.

It is on observations made as to the growth of microzymes in colonies that the little which can be stated as to the *form* in which they originate is based. In the spheroidal masses above referred to, and indeed whenever microzymes occur in a gelatinous matrix which can be distinguished, we observe foci of growth at which the particles are indefinitely minute and spheroidal; around these foci there are zones of matrix, already obsolete and disintegrating, which are inhabited by staff-shaped microzymes of larger size, which eventually become free and display their proper movements. Here therefore it seems probable that bacteria come into distinguishable existence not as rods but as spheroids. Subsequently they multiply, as is well known, by division.

As to the *conditions of their origin* there is even less knowledge and more difference of opinion. There being an immense preponderance of evidence that they do not spring into existence of themselves in the media in which they grow, most observers have looked for germs in the atmosphere, but with no success. Nor has anyone excepting Professor Hallier even suggested a plausible theory on the subject. Liquids which contain no particle distinguishable under the highest powers of the microscope can often (as will be hereafter shown) be proved to possess the property of evolving microzymes without contact with external media, and must therefore contain the germinal substance from which these organisms spring. In interpreting this fact it may be supposed either that the germinal substance is universally and equally distributed, *i. e.* dissolved in such liquids, or that it is unequally distributed or particulate. That any living substance is soluble in water is not at present admissible; we must therefore accept the other alternative, and believe that we have to do with particles so minute that they do not

interfere with the homogeneity of the liquid. In so far as relates to the ultra-microscopical origin of the bacteria, this inference harmonises entirely with what has been stated above as to their development in gelatinous masses from foci. Here, as in the other case, it would surely be an error to suppose that in these proliferous foci the apparently hyaline material is really homogeneous. It appears to be so, merely because the particles are so extremely minute. Hence when we apply the term matrix to this subject, we must guard against the word being understood to imply that in the present instance bacteria arise out of an amorphous jelly. What is meant is, that the jelly is itself so organised throughout, that the smallest conceivable bit of it, if separated from the rest, would still possess structure, and consequently the power of reproduction.

Chemical composition of Microzymes, and their relation to the media in which they grow.—Of the chemical composition of microzymes we know very little. It is assumed that the particles are albuminous, because they are readily stained with carmine and browned by iodine; but of the matrix little can be said, excepting that it is probably also albuminous. Chemistry can as yet give no account of the difference betweeen them. As regards their action on the liquids in which they live the most important facts are: (1) That their growth is attended with absorption of oxygen and discharge of carbonic acid. (2) That they are remarkably independent of the chemical constitution of the medium, provided that they are supplied with oxygen; and (3) That they take nitrogen from almost any source which contains it, and use it for the building up their own protoplasm.

It is this last power which specially indicates what may be called their place in nature as the universal destroyers of nitrogenous substances, acting as the pioneers if not the producers of putrefaction. They exercise this function not by virtue of any special relation of their own nutritive processes to putrefaction as such, but simply by their extraordinary power of seizing on the elements which they require for the construction of their own bodies.

The necessity of oxygen to bacteria is so great that they cannot grow even for a short time without it. Thus if liquid containing living bacteria be placed under a cover glass for microscopical examination, it is seen that towards the centre of the cover glass their movements become sluggish and eventually cease, although towards the edges they are still lively. If bacteria are confined in a tube without air they soon die. If the supply of air is limited they continue to

live only so long as the air to which the liquid is exposed still contains sufficient oxygen. If microzymes exist in great numbers in a liquid, air which has remained for a length of time in contact with it has a large excess of carbonic acid, and occupies less volume than it did originally under the same conditions of pressure and temperature. We have found that in such air a taper is immediately extinguished, whence it would seem that microzymes are able to use up nearly the whole of the oxygen which is supplied to them.

When microzymes grow at the expense of disintegrating organic substance, it cannot be supposed that they avail themselves of the albuminates already existing in it to build up the material of their own bodies. If this were the case it would be impossible to understand the fact that they grow quite as luxuriantly when the nitrogen they require is supplied to them in the form of salts of ammonia, as when it is in the form of ready-made albumen; for clearly it must require a much greater expenditure of plastic energy to build up protoplasm of elements derived from such sources, than merely to convert one albuminous compound into another. It therefore seems probable that bacteria do not use the material on which they feed until it has already been converted by oxidation or by splitting into lower chemical combinations.

The question how far microzymes are the cause of putrefaction will, I think, be elucidated by the results of the following experiments. It will be shown that so long as the germinal matter of microzymes is excluded, animal fluids or tissues withstand decomposition for very long periods, while the slightest contact with media containing this material at once determines septic changes. Consequently it can be asserted positively that under certain circumstances the presence of microzymes excites putrefaction; but the facts do not afford grounds for stating that they are the cause of putrefaction, or that if it were not for them the process would be postponed indefinitely. It is indeed asserted by chemists, and we do not propose to deny, that organic matter may, under the influence of heat and moisture alone, undergo decompositions which present all the chemical characters of putrefaction, even though no microzymes be present.

Method.—As regards the questions which form the principal subject of this report, we at present possess no exact information. As has been already stated, there is a general belief that microzymes exist potentially in the air, and it is also admitted that they may be met with in the blood in certain septic diseases. Hallier, on the other hand, finds

them not only in septic, but in all contagious fluids, while Béchamp imagines that they form part of healthy structures. To determine these questions it was necessary (1) to subject the media to the action of some qualitative test by which the presence of the germinal matter of microzymes could be detected; and (2) to make experiments in which their action on the animal liquids and tissues would be observed under conditions similar to those which exist in the living body. As a test for the presence of microzyme germs we have used first, Pasteur's solution, and secondly animal fluids, either diluted with pure water or undiluted. These liquids were selected on the ground first that they contain nitrogen, in the one case in the form of an ammonia salt, in the other in that of an albuminous compound, and secondly that although transparent and free from visible particles when fresh, they become in a short time peopled with microzymes when kept under ordinary circumstances and at ordinary temperatures. Before using them, however, for the purpose intended, it was necessary to determine that they do not in themselves contain the conditions of evolution; in other words, that they can be prepared and kept in a state of absolute barrenness without prejudice to those qualities by which they are fitted to be employed as tests. These requirements could only be satisfied by a preliminary series of experiments having for their purpose to determine the question of so-called "spontaneous generation," not in general, but with respect to the particular liquids to be used. In approaching a question of such difficulty, even with the limitation above stated, there are two methods of inquiry which suggest themselves; one consists in the comparison of results obtained when the cause to be investigated is present, with those which are produced when it is absent, all other conditions remaining unaltered (method of crucial experiment); the other in the comparison of variations in the results with variations in the circumstances that lead to them (method of concomitant variations). We shall find that the first of these methods, which is clearly the most conclusive, is as applicable as the other to the particular question before us, that of the spontaneous evolution of organic forms in any given medium. But even if it had not been so, the other method would still have been open to us, for if it could be shown that the appearance of microzymes in a given liquid is either delayed or diminished, in a degree proportionate to the degree of exposure to external influences, it might be safely inferred that exposure to the air is the efficient cause of their development. In the present instance it is possible to exclude all conceivable sources of

contamination, and so to obtain a positive answer to the question; but this does not render it the less advantageous to compare the varying effects of contamination with the conditions to which they correspond, for by so doing we acquire a better knowledge of the nature of these causes, and of the means of obviating them.

The experimental results are stated under three headings, according as they relate to the conditions which limit the evolution of organic forms, and particularly microzymes, in test liquids; to their distribution in ordinary water and in most substances; and lastly to their occurrence in the tissues and liquids of the animal body.

While considering myself exclusively answerable for the accuracy of every statement contained in the report, I am anxious that in so far as the investigation has been a successful one, my assistant, Dr. Ferrier, by whom many of the experiments were both planned and carried out, should participate in whatever credit may be accorded to me.

Section 1.—*Experimental Determination of the Conditions which govern the Development of Microzymes in certain Organic Liquids to be used as Tests.*

I.—July 22nd, 1870.—A large number of capillary tubes prepared for the purpose were filled with serum of blood obtained from a guinea pig a few hours before. According to the mode of filling, and the conditions under which they were subsequently placed, the tubes were divided into five batches, designated respectively *a*, *b*, *c*, *d*, and *e*. The tubes *a* were exposed, unsealed, to the air of the laboratory; *b* were hermetically sealed; *c* were sealed, and thereafter placed in the incubator, in which a temperature of about 40° C. was maintained during the whole period of the investigation, with the aid of a Geissler's regulator; *d* were sealed and heated in the oven to 180° C., and thereafter exposed to the air of the laboratory by breaking off one end; *e* were sealed and heated in the same manner as *d*, and then placed in the incubator. The tubes were examined at various periods within a month after they were prepared. Bacteria were found in numbers in *a*, *b*, and *c*, but no organic forms could be detected in *d* and *e*. The remainder of these two batches were therefore preserved for further examination, until the beginning of March, 1871. They then exhibited the appearances always observed under the microscope in superheated serous liquids,[1]

[1] The most remarkable peculiarity of such liquids is that they contain

but on the most scrupulous examination no organic forms could be discovered either in the tubes which had been kept at the ordinary temperature, or in those which had remained in the incubator.

On August 11 the experiments were repeated under similar conditions, with the exception that the serum employed to fill the tubes was first rendered alkaline by the addition of 0·5 per cent. of soda. In this case no organic forms had appeared in any of the tubes at the end of a month, nor could any be discovered afterwards. The superheated tubes were examined with the rest in March, 1871, with the same result. Two quantities of the same serum were kept in glasses side by side in the laboratory, to one of which only, soda had been added in the proportion already mentioned. In the one containing no soda a luxuriant growth of bacteria and leptothrix appeared in a few days, but nothing whatever could be found in the other. Soda, therefore, in the proportion of half per cent., appears to prevent the development of microzymes.

II.—August 24, 1870.—The albumen of a fresh egg was collected in a clean dry test glass, and several tubes of tolerably large size were filled in the ordinary way and hermetically sealed. Some of them which were not heated were kept at the ordinary temperature, others were subjected in the hot-air oven to a temperature of 200° C. All of these tubes were kept until March, 1871, when it was found that the unheated tubes were still perfectly clear, with the exception that on the side which was undermost as the tube lay on the shelf, its internal surface was lined with whitish granular deposit. The liquid showed no other change, and on a microscopical examination no organic forms could be found. The superheated tubes were in this respect in the same condition. From the negative results in the tubes which had not been heated, it might be inferred that white of egg is incapable of maintaining the life of microzymes, but we shall see hereafter that the fact admits of a totally different interpretation.

III.—August 30.—A large number of capillary and other tubes were filled with a solution of sugar, tartrate of ammonia, and yeast ash, according to M. Pasteur's formula, and divided into two batches, designated respectively *a* and *b*. Some of the tubes *a* after having been sealed, were kept either at the ordinary temperature or in the incubator. The rest were left

masses of apparently semi-fluid material resembling oil drops. These masses are of a distinctly yellow colour, and vary indefinitely in size. They are found in superheated liquids immediately after they are prepared.

open and kept in the laboratory. *b* were sealed and raised to a temperature of 200° C. Some of them were afterwards placed in the incubator, others remained at the ordinary temperature. Specimens of *a* and *b* were examined at various periods up to March, 1871. All of the tubes *a* became turbid sooner or later, and were then found to be crowded in different degrees with bacteria and fungi (torula cells and mycelium). When the remaining tubes were finally opened it was found that in many of them gas had been disengaged in such quantity, that when the end of the tube was broken off the liquid was expelled with violence. In others this evidence of increased tension was wanting. On comparative microscopical examination it was found that the liquid in these last, contained no torula cells. *b* were kept till March, 1871, at which time they were found to exhibit no trace of organic life, whether they had been kept in the incubator or at the ordinary temperature.

IV.—August 18.—It has been imagined that the so-called spontaneous evolution of organic forms is materially increased when the air to which the liquid is exposed has a tension much inferior to that of the atmosphere, and conversely that in liquids subjected to pressures greater than that of the atmosphere the development of such forms is arrested. The following experiments were made to test this supposition. Several capillary tubes were filled with fresh serum of blood of a rabbit kept in an ordinary clean glass. These were sealed and placed in a larger glass tube closed at one end, which after having been drawn out at a short distance from its open end was attached thereby to one branch of a T tube by means of a vulcanite junction. The stem of the T tube was then connected with an air pump, and the other branch with a long barometer tube standing vertically in a cup of mercury. The air was then exhausted, and as soon as the mercury had risen in the barometer tube 15 inches, the flame of a blow-pipe was directed against the narrow drawn-out part of the experimental tube, which was thus sealed while the air which it contained had a tension not more than half that of atmospheric air. The tube was then shaken so as to break all the capillary tubes, so that the whole of the liquid which they contained was exposed to the pressure above indicated. It was then kept at the ordinary temperature. Another tube was filled with capillary tubes containing serum, exhausted to 15 inches, and closed hermetically in the same way. It was then placed in the oven and raised to a temperature of 200° C., after which the capillary tubes inside were broken as before, so as to expose the liquid to

superheated air at 15 inches pressure. Both of the tubes were kept until March, 1871. On opening the one which had not been heated, air rushed into it with great force. Its contents had a putrid smell, and the liquid on microscopical examination was found to contain numerous bacteria. When the superheated tube was opened, the ingress of air was equally forcible, but on microscopical examination no trace of organic forms could be discovered.

From this experiment it would appear that diminished tension has no very considerable effect on the process we are studying. It is further evident that the non-appearance of organic forms in superheated liquids cannot be accounted for by supposing that it is attributable either to the relatively large proportion of the liquid, as compared with the volume of the air which is enclosed with it, or to any other circumstance arising from its being contained in so small a receptacle. A third experiment of the same kind was made on August 30th. A number of capillary tubes were filled with Pasteur's solution, and then sealed and introduced into a large tube, closed in the same manner as in the previous experiment. The whole was then subjected to a temperature of 170° C., after which the contained tubes were broken by shaking. On examining the liquid contained in the broken capillary tubes after several months no organic form could be detected.

The above observations (I to IV) show conclusively that no evolution of organisms took place in the superheated liquids, provided that the air with which they were in contact had also been superheated, whether they were kept at an ordinary temperature or at that of the body; and that the effect was not modified, either by the tension of the air or by its quantity as compared with that of the liquid; and it is further shown that in all the experiments, organisms appeared in the same liquids kept under precisely similar conditions, which had not been superheated. Before, however, drawing any further conclusions from these facts it may be inquired, in how far the cause of the non-appearance of organic forms is dependent on the liquids having undergone chemical changes of such a nature as to render them incapable of supporting life, in which case the negative results obtained could not be attributed exclusively to the non-exposure of the liquids to external media. It will be shown in the sequel that this is true as regards microzymes, that is to say, that superheated organic liquids are incapable of supporting the life of these organisms. It is therefore clear that such liquids do not furnish a suitable soil for studying the question we have in view. With respect to fungi, however, the case appears to

be different; for numerous experiments show that superheated liquids, and particularly Pasteur's solution, when freely exposed to the air in a watch glass or an open tube, become eventually covered with tufts of penicillium.

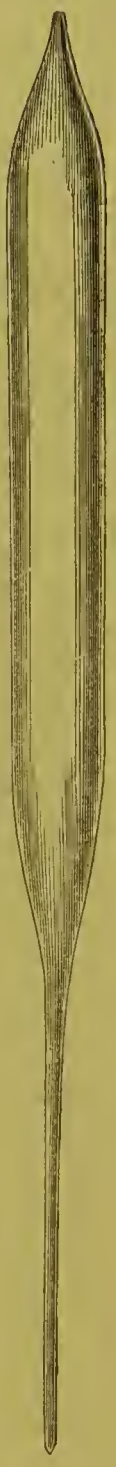

Fig. 1. Tube used in this and the following experiments; the tube is shown in the closed state, and of the actual size.

In the further progress of the inquiry it was found entirely unnecessary to employ so high a temperature as 170° to 200° C. in order to prevent the evolution of organic forms, provided that the liquids were protected from contamination by external media. The experiments which led to this result were as follows:—

V.—August 10.—A number of tubes were filled with serum of rabbits' or sheep's blood. They were then sealed and boiled for an hour or two in a water bath, in consequence of which the liquid contained in several of the tubes became gelatinous, still, however, remaining perfectly transparent. From time to time during the next few months a tube was broken for microscopical examination of its contents, the result being always negative. In March, 1871, the remaining tubes were finally examined. No organic forms could be traced either in those which were gelatinous or in those which remained liquid.

VI.—October 5.—Pasteur's solution was prepared according to formula (the distilled water employed for the purpose being obtained from Messrs. Hopkin and Williams), and placed in a clean capsule. A number of tubes of various sizes were then filled, in the manner already described, with the solution (which had not been heated), and sealed. The solution was then boiled in the capsule for a few minutes, and another batch of tubes were filled in the same manner by breaking their points underneath the surface of the liquid while it was still in a state of ebullition. Each tube was sealed the moment it was withdrawn from the boiling liquid. The two sets of tubes were placed side by side in the laboratory under precisely similar conditions. Some of them were examined microscopically on the 17th. In those of the first batch microzymes in immense numbers, and torula cells, were found along with several filaments of *sporotrichum*. No

organisms whatever existed in any of the tubes containing the boiled liquid. Single tubes of both batches were examined from time to time until March, 1871, the results being always the same. Hence it was concluded that thoroughly boiled liquids, preserved in tubes first prepared and sealed, remain perfectly free from organic forms.

VII.—October 5.—Four tubes, each a quarter of an inch in diameter, were prepared in the usual manner and filled with Pasteur's solution which had not been boiled. Tube *a* was placed vertically in a cork support, its end being truncated so as to expose the upper surface of the liquid to the air. Tube *b* was also placed upright, its upper end having been previously drawn out to a long capillary beak, the tip of which was broken off, so that the interior of the tube communicated with the atmosphere by a small aperture. Tube *c*, of the same form as *b*, was also placed vertically, but its open point was bent downwards at a very acute angle. Tube *d* was sealed at both ends.

Four similar tubes marked respectively *a′*, *b′*, *c′*, *d′*, were then filled with boiling solution and placed side by side with the others, three of them having openings of the characters already described, the other being closed. On October 12 the only change which could be distinguished without the microscope was a very remarkable one. A tuft of penicillium had appeared on the surface of the liquid in tube *b′*, the interior of which communicated with the air only by a capillary aperture. Nothing was visible in the others; but a few days later it was observed that all the open tubes (*a*, *b*, *a′*, *b′*), excepting those of which the ends had been bent down, had similar tufts. In the course of the following six weeks the tufts increased considerably in size. On the 24th of November the liquid in tubes *c* and *d*, in which no penicillium existed, was observed to be hazy and had a slight scum on the surface. Tubes *c′* and *d′* remained perfectly unaltered. The liquid in the open tubes was examined microscopically from time to time during the period of observation, the drop required for this purpose being on each occasion transferred to the object glass of the microscope either by means of a glass rod, the end of which had been first passed through the flame of a Bunsen's burner, or the capillary tube which had been drawn out immediately before, so as to avoid all risk of contaminating the liquid.

In all of the open tubes containing unboiled solution torula cells and microzymes began to appear after the first week. On November 24 they existed in great numbers, in addition to mycelium and filaments and spores of sporotrichum. In the closed tube *d* there were bacteria but no torula or peni-

cillium. At the same date the open tubes *a'* and *b'*, containing boiled solution, were free from microzymes, but contained numerous torula cells and mycelium. *c'* and *d'* were not examined until the 4th of January, at which time both liquids were perfectly clear and contained no organic forms of any description.

VIII.—October 5.—Two test glasses were placed side by side on a shelf under a glass shade, one of which, marked *a*, contained unboiled Pasteur's solution, the other, marked *b*, boiled solution. On October 10 glass *a* was turbid, and was found on microscopical examination to be teeming with bacteria; a thick whitish scum had formed on its surface. Glass *b* was perfectly clear; there were, however, great numbers of torula cells on its surface, but no bacteria. On October 12 *b* exhibited numerous tufts of penicillium, but the liquid still remained limpid and free from bacteria; five days later similar tufts appeared on the surface of *a*.

In the last two experiments it is seen that fungi (torula and penicillium) appeared in unboiled solutions whether they were exposed or not, but much more abundantly when they were exposed than when they were protected; and that in boiled solutions the growth of penicillium was somewhat more luxuriant than in unboiled under similar circumstances of exposure. Microzymes did not appear in the boiled liquids under any circumstances, but were quite as numerous in the tube *d* (Obs. VII), which remained closed for many months, as in any other of the same series. From these facts it seemed clear, not merely that the conditions of origin and growth of microzymes and fungi are considerably different, but that as regards the former the germinal matter from which they spring *does not exist in ordinary air*. The experiments to be next related, however, showed that it would have been wrong to have inferred from these facts that the boiling of a liquid is of itself sufficient to prevent the development in it of these organisms, or that their complete absence in the tubes of the second series of Observation VII (*a'*, *b'*, *c'*, *d'*) was exclusively attributable to this condition.

IX.—October 25.—A solution (A) according to Pasteur's formula, was prepared in the same manner as before, with the exception that water distilled on the same day in the laboratory was used instead of the ordinary distilled water, great care being taken to prevent its contamination. At the same time another solution (B) was made with the same water, of materials which had been previously heated in the hot-air bath to 110° C. Eight glasses were at the same time prepared, of which four, marked severally with the odd num-

bers 1, 3, 1′, and 3′, were washed and dried with a towel. The remainder, numbered 2, 4, 2′, and 4′, were immersed for some time in a vessel of boiling water and then dried as before. The two solutions were then distributed in these glasses as follows:—In 1 and 2 solution A unboiled; in 3 and 4 the same solution after previous boiling; in 1′ and 2′ solution B unboiled; in 3′ and 4′ the same after boiling. Glasses 1, 2, 1′, and 2′ were placed under one shade, and the other four glasses under another. On November 1, tufts of penicillium were obvious on 1, 2, 1′, and 2′, and were beginning to appear on the rest. The liquids were examined microscopically at this date and again on November 8, when the tufts had increased in size. All contained torula cells and mycelium, but microzymes were found only in 1, 3, 1′, and 2′. Thus it appeared that neither the boiling of the liquids, nor of the glasses, nor the superheating of the materials, had exercised any appreciable influence in preventing the development of microzymes. It was still more remarkable that in glass 2, which contained unboiled solution, none of these organisms could be discovered.

These facts, apparently so contradictory, were explained by subsequent experiments.

X.—November 11.—Pasteur's solution was prepared with ordinary distilled water obtained from Messrs. Hopkin and Williams, and distributed in five glasses designated by numbers, the conditions being as follows:—1, a clean test glass, taken from the shelf, was filled without further cleansing with solution which had not been subjected to heat; 2, a similar glass, previously rinsed with distilled water, was filled with the same liquid; 3, a glass just before heated to 200° C. was also filled in like manner. The other two glasses (4 and 5) were charged with boiled liquid, the method used being to boil the solution in a test tube for a few minutes, then to cool it rapidly by dipping it in a stream of cold water, and transfer it at once to the experimental glass. Glass 4 was merely rinsed with distilled water; 5 was previously heated to 200° C. The results were as follows:—On November 20, torula cells were found on the surface of all the liquids. On the 26th, bacteria had appeared in immense numbers in 1, 2, and 4, so that the liquid was milky. In 3 it was apparently clear, but was found on microscopical examination to contain bacteria. Subsequently it also became opalescent. At the same date all the glasses showed tufts of penicillium; those on 3 and 5 were more advanced than the rest, and had become greenish from the development of heads of spores. At this time, and on all subsequent occasions, the liquid in 5 was found to be

perfectly limpid and free from microzymes. The conditions under which the liquid in glass 5 was placed differed from those to which that in glass 4 was subjected in one particular only, viz., in the fact that the former, instead of being rinsed with distilled water and dried, had been superheated. The teaching, therefore, of the experiment was, that the germinal particles from which the microzymes sprung must have been contained either in matter adherent to the surface of the glass, or in the distilled water used to cleanse it, or in both. That the former was not without its influence is rendered probable by the circumstance that in glass 3, which differed from 2 only in having been superheated, bacteria appeared latest. To determine this question was the purpose of the next experiments.

XI.—December 1.—Pasteur's solution was prepared with water obtained from a well at Stevington, in Bedfordshire, which was sent to the laboratory for microscopical examination. The water in question was perfectly limpid; but after it was allowed to stand, a few microzymes could be discovered in the surface layer. None could be detected in the rest of the liquid. It contained a scanty deposit in which one or two *monera* occurred. The solution was distributed in five test glasses, the conditions being as follows:—(1) The solution was boiled in a tube, cooled rapidly, and then poured into a test glass which had just been heated to 200° C.; (2) solution boiled in the same manner was transferred to a glass which had been rinsed and dried; (3) the boiled solution was received in a superheated glass, but just before pouring it in, *the glass was rinsed with ordinary distilled water;* (4) the conditions were exactly the same, excepting that the distilled water used for rinsing *had first been boiled;* (5) the solution was not boiled, but the glass in which it was placed had been previously superheated. The five glasses were numbered in the order in which they have been referred to, and placed under one shade. On December 7, 5 was already milky, the turbidity being due to torula cells and bacteria; 2 and 3 also contained bacteria. On December 8, the turbidity of 5 had increased, and 3 was opalescent. There were no microzymes either in 1 or 4. On the 13th, there were tufts of penicillium on all the liquids; the tufts were more advanced in fructification in 1 and 4 than the rest, but these liquids were still entirely free from microzymes. The last examinations were made on the 21st of December, when 1 and 4 were still in the same condition.

Here the two liquids in which no development of microzymes took place differed from each other in the circumstance

that the glass in which one of them was contained (4) was rinsed with boiled distilled water just before it was charged, both glasses having been superheated. On the other hand, 3, in which microzymes appeared, differed from 4 only in the omission of the boiling of the water used for cleansing. By the comparison of these two results we were enabled to conclude that ordinary distilled water may contain the germinal particles of microzymes in such profusion that even so small a quantity as is introduced into a glass in rinsing is sufficient to render a relatively enormous volume of liquid fruitful. The following is one of a series of experiments which were made to confirm this result:—

XII.—December 3.—Pasteur's solution prepared with the same (Stevington) water was distributed in six glasses, all of which were superheated. Of these three, marked *c*, were filled with solution which had not been boiled, the remainder, *b*, with boiled solution. They were placed in pair's, one of each series in each pair, in different rooms. On December 8, the glasses *c* were all hazy, and found to contain innumerable bacteria, *b* were perfectly transparent; as time went on the contrast became more and more striking in consequence of the increased turbidity of *c*. Subsequently tufts of penicillium appeared on the surfaces of all the glasses, which in this as in the previous experiments progressed more rapidly in the clear solutions than in the others.

This experiment was repeated several times with corresponding results.

In many preceding experiments it has been shown that although torula cells and penicillium appear invariably and without exception on all nutritive liquids of which the surfaces are exposed to the air, without reference to their mode of preparation, no amount of exposure has any effect in determining the evolution of microzymes. This conclusion although it is in complete accordance with what we have already learnt as to their relations both in the visible and invisible state to moisture, is of such importance that it seemed necessary to establish it by special experiments.

XIII.—January 7.—The bent glass tube for the absorption of carbonic acid by potash, known as Liebig's bulbs, was heated to 200° C. and filled with boiling test solution. It was then attached by a vulcanite connecter which had been previously boiled, to an aspirator. During the following week air was drawn through it for a few hours daily. On the 23rd there were numerous torula cells with submerged tufts of mycelium in the liquid, especially in those bulbs to which the air had access first, but no trace of microzymes.

On March 18 the surface of the liquid in the first bulb was crowded with a dense crust of penicillium; in the last bulb there were no tufts, and the liquid was still entirely free from microzymes. The result shows in a most striking manner not only that ordinary air is entirely free from living microzymes, but that the activity of the development of penicillium is in proportion to the degree of exposure.

XIV.—March 2.—A test tube, containing Pasteur's solution, in which there were immense numbers of microzymes and torula cells (penicillium), was plugged with cotton wool, boiled for a few minutes, and placed, still plugged, in a rack, where it remained for some time. The liquid which, at the time of boiling, was quite opalescent, gradually became clear, from the subsidence of the organisms it had contained. It remained perfectly clear and free from organic forms until the 18th of March. The plug of cotton wool was then removed, soon after which tufts of penicillium appeared on its surface; but up to the present time (March 31) the liquid is entirely free from microzymes.

Section II.—*Distribution of the Germial Matter of Microzymes in ordinary Water.*

Having thus found in a number of cases that either contact with surfaces which had not been superheated, or the admixture of water which had not been boiled, was the exclusive cause of the growth of microzymes in the experimental liquid, it was not necessary to go far in order to arrive at the inference that water is the primary source from which the germinal particles of bacteria are derived, whenever they seem to originate spontaneously in organic solutions. To prove this is a number of experiments were made with different varieties of water in ordinary use, in order in the first place to confirm the observations already made, and to ascertain whether all waters possess the properties in question in a like degree.

XV.—January 2, 1871.—A number of eprouvettes of the form shown in the margin were placed in the hot-air oven and heated to 200° C. They were then filled with Pasteur's solution made with ordinary distilled water, under the following conditions:—*a*. Solution not subject to heat. *b*. Solution introduced boiling, which was then allowed to cool; immediately after, a single drop of cold distilled water was added to it. *c*. The same as *b*, with the exception that water from the tap was used instead of distilled water. *d*. The eprouvette was filled with boiled solution, as in *b* and *c*, but

nothing was added to it. The glasses were then carefully plugged with cotton wool. On Jan. 12, glass *a* was quite milky in appearance, and had a gelatinous scum on the surface. It contained myriads of bacteria and a few torula cells. Glasses *b* and *c* were also turbid, the former more than the latter. The microscopical appearances were the same in all. In *d* no change could be detected either by the naked eye or with the microscope. Some of the liquid which remained in a glass exposed to the air was covered with tufts of penicillium.

Fig. 2. Eprouvette used in this and the following experiments. It is plugged with cotton wool and charged with test liquid, to which distilled water has been added in the prescribed proportion, so as to avoid mixing. After standing a week the upper layer, with which distilled water is mixed, has become turbid.

In this experiment, which was confirmatory of the preceding, it is worthy of note that the two waters used to impregnate the test solution the most decided effects were produced by the distilled water.

XVI.—January 17.—Pasteur's solution was made with ordinary distilled water. A sufficient quantity was then boiled, and immediately distributed in four eprouvettes, all of which had been heated immediately before to a temperature of 200° C., the quantity of liquid in each being about equal. The conditions of experiment were as follows:—1. The liquid was allowed to cool, and then three drops of freshly distilled water were added to it with the aid of a small pipette which had just been heated in the flame of a Bunsen's burner. This water was collected in a superheated glass from the glass distilling apparatus, which had been previously thoroughly steamed out. 2. The same, excepting that three drops of ordinary distilled water were used. 3. Three drops of water from the tap were added. To a fourth eprouvette no addition was made.

On January 24 the liquids in 1 and 4 were perfectly limpid, and showed no trace of organic forms; 2 and 3 were milky, especially the former. Both contained bacteria. Up to the present time the eprouvettes 1 and 4 remain perfectly barren.

This experiment shows that if due precautions are taken, distilled water may be obtained in such complete purity that it is free from germinal particles, whether of microzymes or fungi; and that the zymotic power (if I may be permitted to use this term to express the faculty to determine the development of organic forms in a test solution to which it is added)

of ordinary distilled water is aequired after distillation, either by mixture with extremely small quantities of other waters, or by eontact with the surface of the vessels in whieh it is contained. It was also evident that between waters of different kinds and of different sources there are corresponding differences in the degree of zymotic effeet they produce, whence it seemed probable that a praetieal method of judging of the amount of zymotic impurity eontained in any two waters might be founded on the comparison of the degree of opalescenee produced by each in the same time and at the same temperature. In how far this surmise was justified may be judged of by the results of experiments to be hereafter referred to, relating to the zymotic powers of the waters supplied to the metropolis.

If the apparently inevitable contamination of originally pure water, when kept, is due not merely to admixture with other water, but also to contact with surfaees impregnated with living matter, it beeomes of interest to inquire by what conditions the aetion of such surfaces is limited or determined. In the eourse of one of the observations already related it was observed that a boiled liquid eontained in a superheated test glass, whieh had long remained perfectly limpid, and entirely free from organie forms, became turbid after a pipette employed in order to proeure a specimen for microseopieal examination had been dipped in it; and that the time which intervened corresponded with that whieh usually elapses after impregnation before the effect manifests itself. This oecurrenee suggested the following experiments, which were undertaken in order to ascertain how far it is neeessary that a surfaee should be moist in order to its aeting zymotieally.

XVII.—January 30.—A glass rod was eharged with bacteria by dipping it into a solution on the surfaee of which there was a viscous seum, eonsisting entirely of these bodies imbedded in gelatinous matrix. The rod was allowed to dry in the air for a few days; it was then introdueed into boiled test solution contained in a superheated glass. On February 6 the liquid was already milky and teemed with microzymes. On the same day a portion of the same seum was introdueed into a test glass and dried with a gentle heat. The glass was then filled with test solution whieh had just before been boiled and cooled in the usual way. The result was the same as in the previous experiment.

In these instanees it may be readily understood that the drying was very imperfect. To determine the effect of more complete desiceation, an eprouvette containing one cubic centimetre of eold water previously aseertained to be zymotie,

was evaporated to dryness in the incubator and kept for some days at a temperature of 40° C. On February 20 the dried glass was charged with boiled and cooled solution, and plugged with cotton wool in the usual way. The liquid was examined microscopically on March 2, when it contained numerous torula cells, but no trace of microzymes. It therefore appeared that the germinal particles of microzymes are rendered inactive by thorough drying without the application of heat. As, however, it could not be concluded therefrom that drying acted in a similar manner on the microzymes themselves, an experiment was made on this point also.

XVIII.—March 4.—As it appeared probable that in the previous experiments with bacteria scum, desiccation might be prevented by the gelatinous matrix, a portion of the same scum was thoroughly washed with water, collected in an eprouvette, and dried for some days in the incubator. The eprouvette was then (March 4) charged with boiled and cooled Pasteur's solution, and plugged with cotton wool. On March 11, the liquid was slightly hazy, but on microscopical examination was found to contain no trace of microzymes. The haziness was due to the presence of torula cells in great numbers. On the 18th the appearances were similar, but mycelium now existed in addition to torula. It thus appeared that fully formed bacteria are deprived of their power of further development by thorough desiccation; so that we may conclude that the *contamination of water by apparently dry surfaces happens only in those cases in which desiccation is incomplete.* It will be seen that this conclusion is quite consistent with the previous observations.

Method of testing the zymotic property of water.—As a test of the faculty possessed by all water which is not absolutely pure, of determining the growth of microzymes, Pasteur's solution gives results so constant and satisfactory that it appears scarcely necessary to seek for better, although there is no doubt that many other liquids would answer the same purpose, and that some would react with greater delicacy. The method consists, as already indicated, in the addition of a small quantity of the suspected water to a relatively large volume of the solution. As it is very desirable that the conditions of experiment should be subject to as little variation as possible, in our test experiments we add one drop of water to a centimetre of solution, always using the same dropping pipette. As the eprouvettes commonly employed contain five centimetres when half full, this quantity is preferred, so that in the following paragraphs the term "charged eprouvette" is understood to mean an eprouvette which has

been first superheated and then filled to five cubic centimetres with boiling solution. After each testing the pipette is immersed for several minutes in boiling distilled water. In six days after impregnation with any zymotic water such an eprouvette becomes hazy. It need scarcely be added that in each experiment a second charged eprouvette must be placed beside the impregnated one for comparison. Both must be protected from the air by plugs of cotton wool.

From the most careful and repeated examinations of waters known to be zymotic, we have learnt that such waters often contain no elements or particles whatever which can be detected by the microscope; so that it may be concluded that the elements of which the germinal substance of microzymes consists are of extreme minuteness. It therefore appeared to be of great importance to extend our inquiries to water which is optically pure, not merely in the sense that it contains nothing which can be detected by the microscope, but in the much higher sense that when viewed in the electric beam by the method employed by Professor Tyndall it shows no haze. Unfortunately it is not as yet possible to procure such water. Professor Tyndall has, however, been good enough to give us the opportunity of testing specimens obtained by the fusion of ice which approach the standard of optical purity so nearly that the electric beam in passing through them displays a blue colour, Of the results of our examination of these specimens it is sufficient to state that they are as zymotic as many other varieties of water which in the beam are seen to be full of light-scattering particles.

To determine in how far the zymotic properties of water are affected by chemical compounds which are believed to have the power of arresting the evolution of living forms in organic liquids, a series of experiments were made in which the zymotic power of water was tested before and after the addition of such compounds, the supposed anti-zymotic being contained sometimes in the water to be tested, sometimes in the test solution.

XIX.—March 2-10.—1. A quantity of water previously ascertained to be zymotic was ozonised by subjecting it to the action of air which has passed over fresh and moist phosphorus, the apparatus for this purpose consisting of (*a*) two Woulff's bottles containing sticks of phosphorus; (*b*) a washing bottle containing solution of caustic potash; (*c*) a flask containing the water to be ozonised. Air was made to pass slowly through *a*, *b*, *c* in succession, by means of an aspirator, for several hour, after which the liquid in *c*, and the air in contact with it, reacted strongly on iodide of potassium and

starch paper. Charged eprouvettes were then (March 2) prepared in the usual way, to each of which a few drops of the ozonised water was added. On March 21 no organic forms whatever could be discovered in the liquid. The plugs were then removed. On the 27th the first tufts of penicillium appeared, which have increased up to the present time. There are no microzymes in the liquid.

2. Water known to be zymotic was treated with Condy's liquid in quantity sufficient to colour it slightly, A few drops were then (March 2) added to a charged eprouvette. Up to the present time the liquid remains free from microzymes, but contains torula cells. On the same day a second charged eprouvette was treated with two drops of undiluted Condy's liquid and plugged. It remained absolutely barren till March 21, when the plug was removed. In a few days torula cells appeared, and on the 27th there were tufts of penicillium.

3. A charged eprouvette was impregnated (March 2) with ordinary distilled water containing 0·1 per cent. of carbolic acid. At the end of a week the liquid was hazy and teemed with bacteria and torula cells. Ultimately penicillium tufts appeared on the surface. On March 8 the experiment was repeated with water containing 0·5 per cent. of carbolic acid. It remains up to the present moment free from microzymes, but contains torula cells and mycelium.

4. A charged eprouvette was impregnated with ordinary distilled water (March 2) containing 0·1 per cent. of sulphate of quinia, and plugged. At the end of a week it was opalescent and full of bacteria; it also contained torula cells and mycelium. On March 8 the experiment was repeated with water containing 0·5 per cent. of the salt. At the end of a week it was hazy, but on microscopical examination this was found to be due exclusively to torula cells.

5. March 11.—A charged eprouvette was impregnated with distilled water containing 10 per cent. of the solution of peroxide of hydrogen. The liquid remained free from microzymes until March 21, when the plug was removed. Tufts of penicillium had already appeared on the 27th.

6. March 11.—A charged eprouvette was impregnated with distilled water containing five per cent. of liquor chlori. The liquid remained barren until March 21, when the plug was removed. It is now crusted with penicillium.

7. February 13 —A superheated eprouvette was charged with some of the superheated Pasteur's solution which had been prepared five months before. The liquid was then impregnated with distilled water known to be zymotic. On March 4 the liquid was examined and found to be entirely

barren. It was then treated with boiled solution of pure sugar. As on March 21 the liquid was still entirely free from organic forms it was sown with some fresh spores of penicillium. Up to the present time there has been no change.

XX.—February 23.—For the purpose of investigating the zymotic property of any water, it may be conveniently collected in a tube of the form and size shown in Fig. 1. It is essential that it should be used in a state of absolute purity. As it is seldom possible to draw the tube at the time that it is to be filled, it must, as a rule, be prepared beforehand, in which case it is necessary to close it hermetically at both ends before it is removed from the flame. Such a tube obviously contains calcined air, of which the tension is very much less than that of the atmosphere; consequently it is very easily filled by breaking off its end under the surface of the water of which a specimen is to be collected. If the water is flowing from a tap or in a stream it must be received in a boiled capsule, in the contents of which the tube may be dipped.

Fifteen specimens of water supplied by the London companies were collected in this way during February last, and tested with reference to their zymotic power on the 23rd of the same month. The results of the experiment are exhibited in the following table, in which the number printed below the designation of each specimen indicates the order in which it would have stood if the tubes had been arranged in a linear series according to the degree of turbidity which each manifested on the ninth day after impregnation.

Letter designating Water Company.	Water before filtration (from subsidence reservoir).	Water after filtration (from pump well).	Water as distributed (from main).
A	15	3	13
B	14	10	7
C	5	2	6
D	1	12	8
E	4	9	11

The specimens to which the numbers 15, 14, 13, 8, 7, 5, correspond, became hazy as early as the fifth day. On the ninth day it is noted that the eprouvettes to which the six highest numbers correspond were milky, while those in which the turbidity was least marked were merely hazy; the rest

are described as being opalescent. All, therefore, acted zymotically in different degrees.

All of the eprouvettes were plugged with cotton wool. As in previous experiments, the quantity of water introduced was in each case measured with the same pipette, which was immersed in boiling distilled water for a few minutes between each impregnation. A check eprouvette was then impregnated in the same way as the rest with the water in which the pipette was washed. It remains to the present moment perfectly transparent and barren.

Excepting in so far as this experiment shows that filtration exercises no perceptible influence on the zymotic power of water, no conclusion can be drawn from the comparison of the results. It happens that the water designated C stands considerably higher than the rest, and that designated A considerably lower. It would be premature, however, to attach importance to this fact.

Section III.—*Circumstances which Determine the Existence of Microzymes in Organic Liquids and Tissues.*

The experiments to be related in the following paragraphs were undertaken for the purpose of ascertaining whether the tissues and liquids of the living body participate in the zymotic property which has been shown to exist in ordinary water and moist substances: in other words, whether the living matter with which the body is in constant contact by its external surface penetrates into its interior.[1]

XXI.—March 24.—A glass canula of suitable size, which had just been drawn, was introduced into the carotid artery of a rabbit, and secured with a ligature. The arterial blood as it flowed from the canula was received into four ordinary test glasses (marked *a*, *a*, and *b*, *b*), and into an eprouvette (marked *c*). The quantities of blood collected in *a*, *a* were mixed with boiled and cooled distilled water, and left freely exposed under a bell jar. In two or three days bacteria appeared and the liquid became offensive. The quantity in *b*, *b* was left undiluted, and each glass was covered with a layer of cotton wool. On the 30th they remained unaltered,

[1] As to the existence of *visible* microzymes in the liquids of persons affected with contagious diseases, I had already satisfied myself that I could not accept Hallier's observations; for on examining the blood of patients affected with scarlatina (in which, according to Hallier's statement, micrococcus is constantly observed and very abundant) at all stages of the disease, I had found that no such bodies existed in it. It does not, however, follow from this that organisms are not present potentially, *i.e.* in the form of germinal particles not distinguishable by the microscope.

and contained no organic forms excepting those proper to the liquids. They were then carefully mixed with boiled distilled water by the aid of a freshly prepared pipette, and again covered with cotton wool. [On April 3 the liquid was entirely free from microzymes, and exhibited no sign of decomposition.][1] The blood contained in the eprouvette was allowed to coagulate, and yielded a clot and very limpid serum. Up to March 30 it remained quite unaltered, and on microscopical examination it was found to be quite free from microzymes. On that day the serum was transferred by means of a superheated pipette into another superheated eprouvette, and diluted with boiled and cooled distilled water: it was then placed in the incubator. To the clot distilled water was added in quantity corresponding to that of the serum which had been abstracted: it was placed in the incubator. [When these preparations were examined on April 3, the serum was still limpid and perfectly free from organic forms, and the clot-preparation showed no change.]

Other experiments were made, consisting in impregnating charged eprouvettes with drops of blood taken directly from the finger, great care being taken in each case to cleanse the surface of the skin where the puncture was made. In each case the blood-corpuscles subsided to the bottom of the eprouvette, leaving a clear liquid in which no development of microzymes took place, although they were kept under observation for several weeks.

We have no hesitation in attributing the development of bacteria in the liquid in the test glasses marked *a*, *a*, to an accidental contamination (*e.g.* to the falling into the glass of a hair of the rabbit, or possibly a drop of saliva), and in concluding that normal blood contains no microzymes potentially or actually.

XXII.—February 24.—A guinea pig was killed, and, immediately after, the integument was stripped off the back. Portions of the muscles and cellular tissue of the rump were then rapidly cut out with scissors which had just been heated in the flame of a Bunsen's lamp. The pieces were then seized with the aid of glass hooks which had just been made for the purpose, and transferred into charged eprouvettes (marked *a*). Others were placed in superheated test glasses, and covered with boiled and cooled distilled water, but by accident one of them fell from the hook on to the table (marked *b*). The skin was then stripped off the thighs, which were immediately separated from the body with the

[1] The Report bears date March 31st, 1871. The passages in brackets were added during the first week of April.

same precautions as before, and hung up under a bell jar by wires which had been heated and cooled. The liquid in the eprouvette *a* was subjected to repeated examination until March 3, when it was still perfectly limpid and entirely free from organic forms. A single drop of common distilled water was then added to it. In a few days it became milky and acquired a putrid smell. In the glass *b*, there were already signs of bacteria on March 2, and the liquid soon became offensive. The thighs which were hung up, shortly became covered with a crust of penicillium. One of them was examined on March 9. On removing the crust and cutting into the muscle it was found to be less moist, but otherwise of natural appearance. There was a musty but no putrefactive smell. The cut surface was neutral to test paper. The other thigh was examined March 27, and was in a similar condition, excepting that the muscular substance was drier and of darker colour.

XXIII.—February 1.—Five centimetres of urine were introduced into an eprouvette, which was then plugged with cotton wool and placed under a glass shade. It retained its acid reaction and limpidity till February 9, when a drop or two of ordinary cold distilled water was introduced from a fine capillary pipette prepared just before. On the 16th the liquid was hazy and crowded with bacteria. In the course of a few days more, a sediment subsided to the bottom of the eprouvette, and the liquid became alkaline and ammoniacal. This experiment was subsequently repeated with similar results.

It has been long known that the tendency of urine to undergo decomposition may be obviated by protecting it against contamination from without. The preceding experiments show that here, as elsewhere, water is the contaminating agent.

XXIV.—January 2.—An abundant flow of saliva having been determined by introducing a few drops of ether into the mouth, one or two drops were allowed to fall into a charged eprouvette. The liquid was repeatedly examined during the next three weeks, but no microzymes could be detected. The salivary secretion, as it is discharged from the salivary ducts, is no doubt inactive; but inasmuch as the mixed liquid with which the mucous membrane is moistened is exposed to several sources of contamination, and moreover can be often shown to contain leptothrix filaments, it would not have been surprising if the result of the experiment had been otherwise.

XXV.—It is scarcely possible to obtain milk in a state of

purity, for the liquid as it issues is exposed to contamination both from the hands of the milker and from the surface of the test itself. It is not therefore surprising that the results of our experiments with this secretion were not uniform. Their variations, however, exhibit so complete a correspondence with the varying conditions of the experiments, that they are scarcely less confirmatory of the general conclusions we have arrived at than if they had been positive.

February 28.—Milk was received directly from the cow into two flasks (marked *a* and *b*) which had been previously superheated. The flasks were immediately plugged with cotton wool. Another specimen of milk "as delivered to customers," was brought from the dairy at the same time in a clean bottle which had not been superheated. All the specimens were alkaline. On March 4 it was found that the milk in the bottle was slightly acid and crowded with bacteria. On the 9th it was curdled and smelt offensively. The flask *a* was also acid on March 4, and contained a few groups of bacteria. In the flask *b* the acid reaction was scarcely appreciable, and no bacteria could be discovered in it. On the 9th the contrast between *a* and *b* was still very striking, the liquid in *a* having separated into whey and curd, while *b* remained apparently homogeneous. Charged eprouvettes were then impregnated with drops of the liquid in *b* in which no bacteria could be detected. After a few days bacteria appeared in the test liquid, and in the liquid which still remained in the flask.

The difference between *a* and *b* was of course accidental, for both were exposed to equal chances of impregnation.

XXVI.—February 21.—It has been already stated that superheated tubes containing egg albumen which had been kept from August, 1870 to March, 1871, were found absolutely free from organisms, and to all appearance unaltered. The liquid contained in one of these tubes which was perfectly limpid was emptied into a superheated eprouvette and impregnated with two drops of cold distilled water. On March 2 the liquid had acquired a yellowish green tint, a scum had formed on its surface, and the liquid was full of separate bacteria.

XXVII.—March 20.—Pus was collected from a deep-seated abscess in the thigh of a child by introducing the capillary end of a collecting tube into the path of the bistoury which had been used for opening it, the bistoury having been itself immersed in boiling water. It was then transferred to a small eprouvette and exposed to the air. On March 30 there were no bacteria. It was then diluted with

boiled and cooled distilled water. [It was again examined on April 3, when it contained no organic forms whatever.]

February 7.—A pyæmic abscess of the elbow joint was opened; a full stream of pus issued from the incision. Several large but still capillary tubes were then filled by inserting their open ends into the track of the bistoury. The tubes were immediately sealed, and the contents used the same day to impregnate a charged eprouvette. After a few days the test liquid was teeming with bacteria.[1] In this case the knife was not previously immersed in boiling water, but the discharge of pus from the wound was so copious that I do not think there is the slightest doubt that the quantity used was collected without any contamination, whether arising from this source or from the surface of the skin.

XXVIII.—The collection of blister fluid is attended with much greater difficulties than that of pus, for it is almost impossible to abstract it from the vesicles without risk of contact with the surface of the skin. It can be best obtained by opening both ends of a collecting tube, and then introducing the capillary end into a vesicle after first snipping the epidermis. This done, the liquid must be drawn into the tube by suction. Liquid thus collected was used as follows:—

January 10.—Blister fluid was added in the usual proportion (one drop to one cubic centimetre) to a charged eprouvette. For a long time the liquid remained clear, but eventually bacteria appeared in small numbers.

February 13.—Blister fluid from another source was used in a similar manner and with a similar result.

March 27.—The same experiment was repeated with different fluid, but in this case the eprouvette was kept in the incubator. The development of bacteria was much more rapid. On the same day another quantity of the same liquid was diluted with boiled and cooled distilled water in a superheated eprouvette and also placed in the incubator. [In a few days it became turbid and swarmed with bacteria.]

The equivocal results of these experiments are to be attributed entirely to the difficulty of obtaining blister fluid pure, that is, to accidental contamination in the process of collection.

---

[1] This important experiment could not be repeated, for an attendant who entered the laboratory in my absence carelessly destroyed all the tubes excepting the one which had been already used. The single result was so satisfactory that I myself entertained no doubt of its significance.

From the consideration of a number of facts which presented themselves in the course of the experiments related in the previous pages, it has appeared certain that there is no developmental connection between microzymes and torula cells, and that their apparent association is one of mere juxtaposition. The grounds of this conclusion may be shortly stated thus:—

1. The prompt appearance of torula cells in Pasteur's solution whenever it is exposed to the air, and the rapid development and luxuriant fructification of the higher form (penicillium), show that so far as the chemical composition of the liquid is concerned, there exist in it all the conditions favourable to the process.

2. Our experiments prove that when precautions are taken to prevent contamination by impure surfaces or liquids, the development which ends in penicillium goes on from first to last without the appearance of microzymes.

3. Whenever it is possible to impregnate the test liquid with microzymes without at the same time introducing torula cells or germs, the development of the former begins and continues by itself without any transformation into the latter.

Thus fungi are not developed, *notwithstanding the presence of microzymes* in the same liquid in which, *microzymes being absent*, but air having access, they appear with the greatest readiness.

This being the case we are enabled to eliminate the question of the quasi spontaneous evolution of fungi altogether in the present discussion, as lying beyond the limits of our inquiry. It can hardly, however, be considered out of place to state to the reader some of the results to which our observations have led us with reference to this question, especially considering that however improbable it may seem to ourselves that fungi have any important relation with the processes of disease, there are others who are of a different opinion.

To determine the forms in which germs of fungi exist in the air, the best method is that long ago used by Pouchet—that of projecting a jet of air on a glass plate moistened with glycerine or syrup. A few experiments were made, but the results were mostly negative, for in London the particles of soot and refuse fragments which are collected by this method are so numerous that organised particles, even if present, could scarcely be distinguished. We find it a much more successful plan simply to expose a glass surface covered with glycerine to the air. In examining such a surface it was always possible to discover a certain number of cells

which resembled torula cells, and occasionally penicillium acrospores.

From this result we do not, however, conclude that it is by these forms that the cosmopolitan fungus (as Hallier calls it) is usually propagated; it frequently happens that liquids which have been once exposed, although they contain no visible cells whatever, rapidly germinate without further exposure. We are also certain that although air is the main source of what we may venture to call fungus impregnation, as distinguished from impregnation with microzymes, yet the two acts may take place at the same moment—germs of torula being often contained in the same liquid media as the germ particles of microzymes. That this is so is proved by instances already referred to, in which liquids protected from air filled with torula cells. Here we relinquish this question, although in a biological point of view it is of the greatest interest and importance.
