V. *Further Researches on the Deportment and Vital Persistence of Putrefactive and Infective Organisms from a Physical point of View.* By John Tyndall, *D.C.L., LL.D., F.R.S.*

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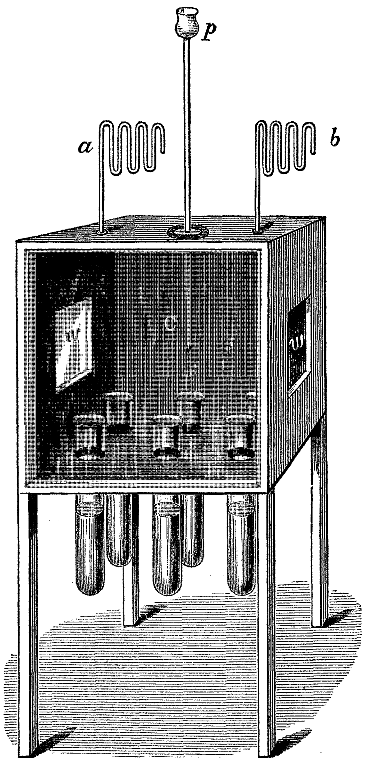
§ 1. *Introduction.*

On the 13th of January, 1876, I had the honour of submitting to the Royal Society some account of an investigation in which the power of atmospheric air to produce life in organic infusions and its power to scatter light were shown to go hand in hand. The "scattering" was proved to be due, not to the air itself, but to foreign matter suspended in the air. It was moreover proved that air placed under proper conditions went through a process of self-purification, and that, when this purification was visibly complete, the power to scatter light and to generate life disappeared together.

Fig. 1.



The form of the experiments here referred to was, it will be remembered, as follows:—Wooden chambers were constructed with glass fronts, side windows, and back doors. Through the bottoms of the chambers test-tubes passed air-tight, their open ends, for about one fifth of the length of the tubes, being within the chambers. Provision was made for a connexion through sinuous channels between the outer and the inner air. The chambers being closely sealed, were permitted to remain undisturbed for a few days. The floating matter of the internal air gradually subsided, until at length an intensely luminous beam failed to show its track within the chamber. Then, and not till then, were the infusions introduced, by means of a pipette passing through the top of the chamber. After their introduction, they were boiled in an oil- or brine-bath* for five minutes, and afterwards placed permanently in a warm room.

The annexed woodcut, taken from the 'Proceedings' of the Royal Institution, shows a chamber with its six test-

* From the fact of their being boiled in oil or brine, Prof. Cohn has inadvertently inferred that the infusions themselves were raised above their boiling-points. The tubes being open, the temperature of ebullition is of course independent of the source which provokes it.

tubes, its side windows *w w*, its pipette C, and its bent tubes *a b*, which connect the air of the chamber with the external air.

In upwards of fifty chambers thus constructed, many of them used more than once, it was, without exception, proved that the sterilized infusion in contact with air shown to be self-cleansed by the luminous beam remained sterile. Never, in a single unexplained instance, did such an infusion show any signs of life. That the observed sterility was not due to any lack of nutritive power in the infusion, was proved by opening the back door and permitting the uncleansed air to enter the chamber. The contact of the floating matter with the infusions was invariably followed by the development of life. Numerous examples of these results were placed before the Fellows of the Royal Society at their Meeting on the 13th of January, 1876.

Prior to the date here referred to, great public interest had been excited, and, I may add, considerable scientific uncertainty had been produced in reference to this subject, both in England and America, by the writings of Dr. BASTIAN. These writings consisted, in part, of theoretic considerations and reflections, not new, but sometimes very ably stated, based on the general doctrine of Evolution, and, in part, of very pungent criticisms of those who, though believers in Evolution, declined to accept the writer's programme of its operations*. Passing over both theory and criticism, I thought it wise to fix upon certain well-defined statements of fact which lay at the basis of the weighty superstructure raised by their author, and to bring these statements to the test of strict experiment.

Thus it was affirmed "that boiled turnip- or hay-infusions exposed to ordinary air, exposed to filtered air, to calcined air, or shut off altogether from contact with air, are more or less prone to swarm with *Bacteria* and *Vibriones* in the course of from two to six days"†. I resorted accordingly to filtered air, calcined air, and to infusions withdrawn from air, but failed to discover the alleged "proneness" to run into living forms. It had also been affirmed that infusions of muscle, kidney, or liver, placed "in a flask whose neck is drawn out and narrowed in the blowpipe flame, boiled, sealed during ebullition, and kept in a warm place, swarmed after a variable time with *Bacteria* and allied organisms"‡. I resorted to such flasks, employing infusions of fish, flesh, fowl, and viscera, and on the 13th of January was able to place before the Royal Society one hundred and thirty of them, every one of which negatived the foregoing statement.

Two objections were subsequently urged against these results. The infusions, it was contended, were not sufficiently concentrated, nor were the temperatures sufficiently high. Both these objections were met by the statement that forty-eight hours' exposure under the same circumstances to common air sufficed to fill these same infusions with life. Beyond this, however, I was able to show that the temperatures employed by me were exactly those which had previously been found most effectual by the writer who urged

* See 'Evolution, or the Origin of Life,' pp. 168, 169.

† Evolution, p. 94.

‡ Transactions of the Pathological Society, 1875, p. 272.

the objection. Other temperatures, higher than any previously employed, were at the same time said to ensure spontaneous generation. I exposed my infusions to these newly discovered efficient temperatures, but found that they remained as barren as before.

With regard, moreover, to the question of concentration, it was shown that, owing to their gradual vaporization, the infusions used by me were probably unequalled in strength by those employed by any previous investigator. Some of these infusions remain with me to the present hour. Concentrated by twelve months' slow evaporation, and reduced to one fifth of their primitive volume, they still exhibit the purity of distilled water.

These results have been published in the Philosophical Transactions, and Dr. BASTIAN has made no attempt to invalidate them. They prove beyond a doubt that in the atmospheric conditions existing in the laboratory of the Royal Institution during the autumn, winter, and spring of 1875–76, five minutes' boiling sufficed to sterilize organic liquids of the most diverse kinds. Among these may be mentioned urine in its natural condition, infusions of mutton, beef, pork, hay, turnip, haddock, sole, salmon, cod-fish, turbot, mullet, herring, eel, oyster, whiting, liver, kidney, hare, rabbit, barn-door fowl, grouse, and pheasant. Once properly sterilized, and protected afterwards from the floating matter of the air, not one of these putrescible infusions ever manifested the power of generating by its own inherent energy putrefactive organisms of any kind.

§ 2. *Experiments of* PASTEUR, ROBERTS, *and* COHN.

During the investigation just referred to I confined myself for the most part to animal and vegetable juices in their natural condition—that is to say, extracted by distilled water, and not rendered artificially acid, neutral, or alkaline. I had occasion, however, to repeat among others some of the very remarkable experiments on superneutralized hay-infusions described by Dr. WM. ROBERTS in his excellent paper in the Philosophical Transactions for 1874. These experiments I could not corroborate; for while in his hands such infusions sometimes required three hours' boiling to sterilize them, in mine they behaved like other infusions, and were sterilized in five minutes.

In the abstract of the investigation communicated to the Royal Society on the 13th of January, 1876, I mentioned this discrepancy, and pointed out its possible cause *. But the largeness of the question, which had been long previously raised by M. PASTEUR, and the limitation of my time led me to postpone it. This postponement is mentioned at the conclusion of my paper in the Philosophical Transactions, where the discrepancy referred to is not at all discussed.

In his celebrated paper, "Sur les corpuscules organisés qui existent dans l'Atmosphère," published fifteen years ago †, M. PASTEUR first announced that while acid infusions had their germinal life destroyed by a temperature of 100° C., a temperature over 100° was needed to produce the same effect in alkaline infusions. In his 'Études sur la Bière,' published in the early part of 1876, he repeats and illustrates this statement.

* Roy. Soc. Proc. vol. xxiv. p. 178.

† Annales de Chimie, 1862, vol. lxiv.

Vinegar he finds has the organisms which decompose it destroyed by a temperature of 50° C. Wine is rendered unchangeable by a slightly higher temperature. Beer-wort without hops requires a temperature of 90° C. to sterilize it, and milk a temperature of 110°. Fresh urine has its organisms destroyed at a temperature of 100°, while a higher temperature is needed when the urine has been neutralized by carbonate of lime*. The resistance of alkalized urine to sterilization is therefore by no means a new announcement†.

On my return from Switzerland last autumn the experiments on alkalized hay-infusions were resumed; and soon afterwards Professor COHN, of Breslau, so highly distinguished by his researches on *Bacteria*, placed in my hands a memoir‡ which rendered it doubly incumbent on me to examine more strictly the grounds of my dissidence from Dr. ROBERTS. Professor COHN is emphatic in his corroboration of Dr. ROBERTS§, having found, during a long and varied series of experiments with hay-infusions of divers kinds, that when the period of boiling did not exceed fifteen minutes organisms invariably appeared in the infusions afterwards. Sixty, eighty, and even one hundred and twenty minutes' boiling were found in some cases insufficient to sterilize the infusions. One marked difference, however, exists between Dr. ROBERTS and Professor COHN. The former found five minutes' boiling sufficient to sterilize unneutralized hay-infusion, but one, two, and even three hours' boiling insufficient to sterilize superneutralized hay-infusion; while the latter noticed no difference of this kind, but found acid and neutral infusions equally resistant‖.

* Études sur la Bière, p. 34.

† With regard to the different action of acid and alkaline liquids, I put the subject purposely aside with the view to its full investigation as soon as the first instalment of these researches had been published. I could find no adequate explanation of the alleged fact that germs are killed in an acid liquid, while they survive in an alkaline one of the same temperature; nor could the well-merited respect that I feel for M. PASTEUR cause me to accept his explanation of the fact without further inquiry on my own account. In due time, therefore, I resolved to examine the question. Various experiments and explanatory views regarding it are recorded in the following pages. It is perhaps worth mentioning that in his communication to the Academy of Sciences Dr. BASTIAN so interprets my last paper (Phil. Trans. 1876, p. 57) as to make me say that which I had neither the warrant nor the wish to say—namely, that germs are killed in alkaline liquids of all kinds by one or two minutes' exposure to a temperature of 212° F.

‡ Beiträge zur Biologie der Pflanzen, July 1876.

§ Professor COHN gently censures me for taking exception to the cotton-wool plug, seeing that cotton-wool, even in my own experiments, has always proved a trustworthy filter. I did not, however, object to it as a filter, but on grounds which have in part, at all events, commended themselves to Professor COHN himself. With reference to the method of Dr. ROBERTS he writes thus:—"The defect of this method consists in the difficulty of protecting the cotton-wool from accidental wetting by the infusion. The steam, moreover, which rises from the liquid penetrates the cotton-wool, and, through its partial condensation in the neck of the bulb, might readily charge itself with germs."

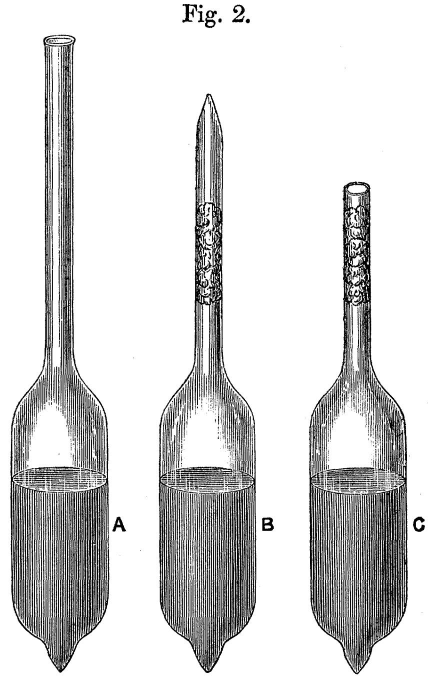
‖ "Ein constanter Unterschied in der Zeitdauer zwischen sauren und neutralen Aufgüssen, wie ihn ROBERTS gefunden, trat in unseren Versuchen nicht hervor" (p. 259).

§ 3. *Hay-infusions. Preliminary Experiments with Pipette-bulbs.*

I have now the honour to submit to the Royal Society an investigation which embraces among others the points here referred to, and which has proved far more difficult and laborious than I expected it would be. On the 27th of September, 1876, a quantity of chopped hay was digested for three hours and a half in distilled water maintained at a temperature of 120° Fahr. The infusion was afterwards poured off, and its specific gravity reduced to the exact figure given by Dr. ROBERTS, viz. 1006. It was then filtered and slightly superneutralized. Precipitation occurred on the addition of the potash, and the infusion was boiled for five minutes to render the precipitation complete. It was then refiltered, and introduced into a series of bulbs of the same size and character as those described by Dr. ROBERTS, and called by him "plugged bulbs"*.

Each bulb was a cylinder about four inches high and upwards of an inch wide, with a long neck attached to it †, as shown at A, fig. 2. Two thirds of the cylinder were occupied by the infusion. After the introduction of the latter, the neck of the bulb was plugged with cotton-wool, and hermetically sealed above the plug, as at B, fig. 2. The bulbs were afterwards plunged in water deep enough to cover their necks, which was gradually raised to the boiling-point, and maintained at the boiling temperature for ten minutes. They were then removed and permitted to cool; after which the sealed end of each neck was broken off by means of a file, its subsequent appearance being shown at C, fig. 2. The bulbs, protected by the cotton-wool plugs in the neck above them, were then exposed to a tolerably uniform temperature of about 90° Fahr.

Fig. 2.



At the same time two similar bulbs, charged with the same infusion, had their necks bent downwards, as in fig. 3 (p. 6), the inclined portion being plugged, so that no impurity could fall into the liquid from the cotton-wool. These two bulbs were boiled for five minutes in an oil-bath, and plugged while boiling with cotton-wool. They were then sealed behind the plugs and permitted to cool, their sealed ends being broken off afterwards.

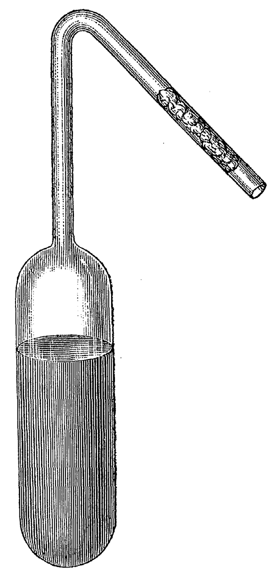
On the 30th of September the infusion in all the straight-necked bulbs was turbid, while in the two bent-necked ones it was perfectly clear. On the 2nd of October

* Phil. Trans. vol. clxiv. p. 460.

† I have called them pipette-bulbs because they are formed by hermetically sealing one shank of a pipette, close to the bulb, leaving the other shank open for the introduction of the infusions. German pipettes, on account of their cheapness, were at first commonly used; but in cases of long-continued boiling, explosions were so frequent that bulbs of English glass of specially resistant quality were resorted to.

the turbidity of the straight-necked bulbs had increased, while a fatty scum had formed on the surface of each. The two others were at the same time slightly but distinctly turbid.

Fig. 3.



My inference from this experiment was that in neither the straight-necked nor the bent-necked bulbs had the germs been killed by the boiling, but that they were more damaged in the former than in the latter.

It is here to be noted that a quantity of air, with its associated floating matter, was imprisoned above the infusion in every straight-necked bulb; that in the case of the two bent-necked bulbs this air had been in part displaced by steam, the air which entered on cooling being sifted by the cotton-wool plugs. To this difference of treatment is to be attributed the observed difference of deportment. Unlike the thick cloudiness of their neighbours, the turbidity of the bent-necked bulbs, though distinct, was barely sensible, and in none of them was any scum ever formed upon the surface of the infusion.

Examined microscopically, numerous Vibrios were found in the infusions of the straight-necked bulbs, many of them broken at the centre, with the two halves apparently trying to separate from each other. There were also numerous smaller *Bacteria*, very active and of various lengths. In the bent-necked bulbs a number of exceedingly small *Bacteria* were found, but no Vibrios.

The deportment of the hay-infusion employed in these experiments corroborates the results of Dr. ROBERTS *and Professor* COHN.

On the 2nd of October another infusion of hay was prepared, and, after neutralization with caustic potash, was introduced into six pipette-bulbs with straight necks. The necks, being first plugged with cotton-wool, were afterwards sealed by the blowpipe. The infusions were maintained for ten minutes at the temperature of boiling water. Their sealed ends were afterwards broken off, and they were subjected, like the former ones, to a temperature of 90° Fahr.

Six other bulbs were charged at the same time with the same infusion; but instead of being hermetically sealed, they were placed in an oil-bath, and boiled there for five minutes. Before the ebullition ceased, the neck of each was stopped with a plug of cotton-wool.

Up to October 6th all the bulbs continued clear. On the 6th one bulb of the series last described became turbid, lighter in colour than its neighbours, and covered with a fatty scum. On the 7th one tube of the first series (boiled after the fashion of ROBERTS for ten minutes) also became turbid and exhibited the same fatty scum. The remaining ten bulbs maintained permanently their deep brown-sherry colour, their high transparency, and their perfect freedom from Bacterial life. They are still clear, though seven months have elapsed since their preparation.

In the great majority of these experiments the deportment of alkalized hay-infusion contradicts that observed by Dr. ROBERTS *and Professor* COHN.

Six other pipette-bulbs, with their necks so bent and plugged with cotton-wool and asbestos that no impurity falling from the plug could reach the infusion, were also charged on the 2nd of October. Three of the bulbs, with their necks hermetically sealed, were maintained for ten minutes at the temperature of boiling water, the sealed ends being afterwards broken off. The three other bulbs were boiled in an oil-bath, and had their necks plugged before ebullition ceased. All six bulbs have remained perfectly transparent up to the present time.

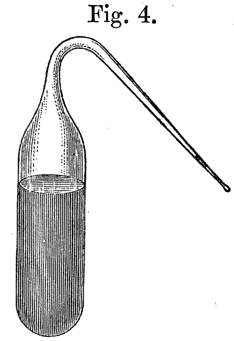
Here, again, we have discordance between my results and those of Dr. ROBERTS *and Professor* COHN.

But on the 6th of October another infusion was prepared and neutralized, exactly in the same fashion as before. Five pipette-bulbs were charged with it; they were hermetically sealed and immersed for ten minutes in boiling water. The sealed ends were afterwards broken off, and the bulbs exposed to a temperature of 90° Fahr. On the morning of the 8th of October (that is to say, two days after their preparation) the infusion in every one of the bulbs was turbid and covered with scum.

Here once more we have perfect harmony between my results and those of Dr. ROBERTS *and Professor* COHN.

Reverting to the 2nd of October, fourteen of our ordinary small retort-flasks with bent necks (shown in fig. 4) were then charged with the neutralized hay-infusion. They were boiled for three minutes, and hermetically sealed whilst boiling. Some days afterwards one tube of the entire number was observed to have become lighter in colour and sensibly cloudy; but thirteen out of the fourteen remained unchanged in colour, brightly transparent, and entirely free from life.

Fig. 4.



Here the dissidence between my results and those of Professor COHN, *who also experimented with hermetically sealed flasks, reappears.*

Numerous other experiments with pipette-bulbs and retort-flasks were made at the time here referred to, but it is unnecessary to record them. Suffice it to say that, like those just described, some of them corroborated and some of them contradicted the results of Dr. ROBERTS and Professor COHN.

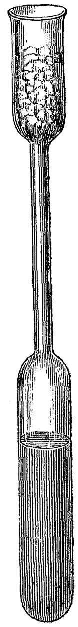
§ 4. *Hay-infusions. Experiments with* COHN'S *Tubes.*

For reasons given by himself*, Professor COHN deviated from the method of experiment pursued by Dr. ROBERTS, employing, instead of the pipette-bulbs, flasks, the nature of which will be understood from the following description. Let a zone of a common test-tube, about one third of its length from its open end, be softened by heat, and let the softened glass be drawn out so as to form a tube of much narrower bore than the original test-tube. Thus modified, the tube would consist of an elongated bulb below

* Beiträge, July 1876, p. 256.

and an open funnel above, both being connected by a narrow neck (see fig. 5). Professor COHN filled the elongated bulb to about two thirds of its volume with hay-infusion, plunged his bulbs in water, raised the water to ebullition, and continued the boiling for the required time. The tubes were then removed from their bath, and after being held open for a minute or two so as to allow the water condensed in their necks to evaporate, the funnel was plugged with cotton-wool.

Fig. 5.



Professor COHN considers that all possibility of external contamination is here shut out*. By his method, therefore, I wished to check the results above described. Accordingly, on the 24th of October, I had four groups of COHN'S tubes (twelve in a group) carefully charged with two freshly prepared hay-infusions. Each infusion was divided into two equal parts, one of which was neutralized and the other left in its natural acid condition. Twelve of the tubes were charged with one of the infusions neutralized, and twelve with the same infusion unneutralized. We will label this infusion A. Twelve other tubes were charged with the second infusion neutralized, and twelve with it unneutralized. We will call this infusion B. The forty-eight tubes were subsequently boiled for ten minutes in tin vessels containing water deep enough nearly to submerge them. Having proved by previous experiments that it was dangerous if not fatal to exactness to expose the infusions for one or two minutes to the air after their removal from the water, I took the precaution of plugging them first and removing them afterwards.

On the 28th of October (that is to say, four days after their preparation) several of the tubes containing the unneutralized infusion A were faintly but distinctly turbid and thinly covered with scum. The twelve neutralized tubes of the same infusion were at the same time perfectly clear. This retarding influence of the alkali has been of frequent occurrence in this inquiry. That it was simply a case of retardation was proved by the fact that, on the 30th of October, the twenty-four tubes, both neutral and acid, of infusion A were turbid and covered with scum.

On the same date the twelve neutralized tubes of infusion B were perfectly clear and without a trace of scum. Of the twelve unneutralized tubes three had given way, and a fourth yielded on the 31st. Four days later three of the neutralized tubes also yielded. The permanent state of matters was that eight out of the twenty-four tubes charged with infusion B had become turbid, while sixteen of them remained perfectly clear. I do not doubt that the tardy infection of some of the tubes just referred to arose from external contamination, which is almost inseparable from the method of experiment.

Here, while infusion A *corroborated Professor* COHN, *infusion* B *in substance contradicted him.*

* "Ehe ich über die Organismen berichte, welche sich in den gekochten Aufgüssen entwickelten, will ich bemerken, dass an eine nachträgliche Infection derselben durch von aussen nach dem Kochen eingeschleppte Keime bei unseren Versuchen nicht zu denken ist" (p. 259). I may remark that, with an atmosphere like that in which my recent experiments were conducted, there would be no chance of escape for an infusion thus handled.

§ 5. *Hay-infusions (in Closed Chambers).*

In dealing with hay-infusions I also fell back on the method of experiment which was found so effectual last year*, employing closed chambers in which the air had been permitted to cleanse itself by the gradual subsidence of its floating matter.

On the 3rd of October, 1876, my experiments with such chambers recommenced. Two of them, containing each three large test-tubes, were then charged with an infusion of hay accurately prepared according to the prescription of Dr. ROBERTS. Its specific gravity was 1006; it was superneutralized to the proper extent with caustic potash, but the period of boiling, instead of being three hours, was five minutes.

Examined from time to time for more than four months subsequently, the infusion in both chambers continued perfectly unchanged. It was free from suspended matter, free also from every trace of scum, maintaining for the light which passed through it a singular transparency.

Here, to a certainty, a period of boiling not amounting to one twentieth of that required by Dr. ROBERTS sufficed to destroy totally the power of generating life in an alkalized hay-infusion.

This result is in perfect harmony with *all* the results of last year. Chamber after chamber was then charged with infusions of hay, which were afterwards subjected to the boiling temperature for five minutes. In every chamber the infusion remained perfectly clear until purposely infected from without. There was no instance observed last year in which five minutes' boiling failed to sterilize hay-infusion, whether neutralized or unneutralized.

Thus, on the 26th of November, 1875, a group of three test-tubes was charged with hay-infusion of the same specific gravity and of the same degree of alkalinity as that found most resistant by Dr. ROBERTS. They were protected by glass shades, the air within the shade being calcined by an incandescent platinum wire in the manner described in my last paper†. The tubes were boiled for five minutes, the subsequent intrusion of contaminated air being prevented by a ring of cotton-wool. Thirteen months afterwards the infusion, greatly concentrated by evaporation, exhibited its pristine deep transparency. A second similar group of tubes was charged with alkalized hay-infusion on the 27th of last January, and on the 5th of December (that is to say, after a period of more than ten months) the infusion was found perfectly clear.

A number of hermetically sealed tubes charged with the same infusion, and boiled for only three minutes, have maintained for more than a year both their primitive transparency and their water-hammer sound. Thus many of the earliest experiments of the present year and the whole body of last year's experiments are in complete harmony with each other.

This harmony was, however, disturbed by some of the foregoing experiments with bulbs and tubes, and it was soon to be further disturbed by experiments with closed chambers. On the 6th of October, 1876, for example, an infusion was got ready in

* Briefly described in the Introduction.

† Phil. Trans. vol. clxvi. p. 50, and § 12 of this memoir.

strict imitation of that prepared on the 3rd; it was of the same specific gravity, it was alkaline to the same degree, and it was introduced in the same manner into a chamber of three tubes; but whereas the infusion of the 3rd remained intact for months, and would have remained so indefinitely, a week had not elapsed before every tube of this new infusion was turbid and covered with fatty scum.

§ 6. *Desiccation of Germs. New hay and old.*

In his work entitled ' Evolution, and the Origin of Life,' Dr. BASTIAN affirms, with repeated emphasis, that living matter is unable to maintain its life when exposed to a temperature even below that of boiling water. He refers to the scalding of the hand and other destructive effects, and also to the action of boiling water on eggs. He also refers to the experiments of SPALLANZANI on seeds, and extends the results observed with living matter of these special kinds to living matter generally. "It has been shown," he writes*, "and is believed by the great majority of biologists, that the briefest exposure to the influence of boiling water (212° F.) is destructive of all living matter." But scientific literature is not without examples which invalidate the inference drawn by Dr. BASTIAN from his special illustrations.

More than ten years ago an extremely significant observation directly bearing upon this subject was made by the wool-staplers of Elbœuf in France. They were accustomed to receive dirty fleeces from Brazil, and among other matters entangled in the wool were the seeds of a certain plant called *Medicago*. It had been repeatedly found by the wool-cleaners that these seeds sometimes germinated after a period of four hours' boiling. The late M. POUCHET repeated the experiment. He collected the seeds, boiled them for four hours, and sowed them afterwards in proper earth. To his astonishment they proved fruitful. He then closely examined the boiled seeds, and found the great majority of them swollen and disorganized; but amongst these ruined seeds he observed others which had refused to imbibe the water or to swell or break up in any way. These he carefully picked out, and sowed them and their neighbours separately in the same kind of earth. The swollen seeds were incapable of germination, while the unaltered ones rapidly gave birth to a crop. This was the only instance of such resistance known to POUCHET when he communicated the fact to the Paris Academy of Sciences.

The observation here described stands recorded in the 'Comptes Rendus' for 1866, vol. lxiii. p. 939, and it subverts the arguments founded by Dr. BASTIAN on the particular cases which he has adduced. It is not difficult, indeed, to see that the surface of a seed or germ may be so affected by desiccation and other causes as practically to prevent contact between it and a surrounding liquid†. The body of a germ, moreover, may be so indurated by time and dryness as to resist powerfully the insinuation of water

* 'Evolution,' p. 46.

† In this connexion a remark of Dr. ROBERTS regarding the resistance of chopped green vegetables merits quotation. "The singular resistance of green vegetables to sterilization appears to be due to some peculiarity of the surface, perhaps their smooth glistening epidermis, which prevented complete wetting of their surfaces."

between its constituent molecules. It would be difficult to cause such a germ to imbibe the moisture necessary to produce the swelling and softening which precede its destruction in a liquid of high temperature.

In my last paper I made some remarks upon this subject *; and in relation to our present experiments, the influence of drying and hardening was brought home to me by the fact that in all the foregoing cases the infusions which five minutes' boiling proved sufficient to sterilize *were, without exception, derived from fresh hay mown in* 1876, *while the infusions which five minutes' boiling failed to sterilize were derived, without exception, from old hay mown either in* 1875 *or some previous year.*

In the earlier experiments of the present inquiry this distinction between old and new hay came most clearly and definitely out. The result was subsequently blurred by circumstances which it required time and labour to unravel, and which will require patience on the reader's part if he would follow them through all their monotonous obstructiveness. They will, however, throw far more light upon the real character of these inquiries, and do more to reconcile the discords to which they have given birth, than if every experiment had been a success unshaded by doubt.

§ 7. *Hay-infusions. Further experiments with Closed Chambers.*

With a view to probing to the uttermost this question of drying and hardening, on the 6th of October an extensive series of experiments with closed chambers was begun. Three different kinds of hay were employed:—1st, Old hay, from Heathfield, Sussex †; 2nd, new hay from Heathfield (both, it may be stated, from a somewhat ungenerous soil); 3rd, new hay purchased in London, and artificially dried for some days upon a sand-bath. For these experiments eleven closed chambers were prepared, as I wished every result to be based as far as possible upon the testimony of two chambers. On the 6th of last October they were carefully charged with the infusions, the period of boiling afterwards being five minutes.

Two chambers were devoted to the acid and two to the alkalized infusion of old hay. Two chambers were also devoted to the acid and two to the alkalized infusion of dried hay. Two chambers were finally devoted to the alkalized and one to the natural acid infusion of new Heathfield hay.

Examined from day to day, differences were soon observed, not only between the different infusions, but also between different chambers containing the same infusion. Thus every tube of both the chambers containing the neutralized infusion of old hay became turbid, but the three tubes of the one chamber were loaded in four days with a fatty scum, while the tubes of the other chamber remained for ten days perfectly free from scum. The two chambers containing the acid infusion of old hay exhibited similar differences. Every tube in both of them became turbid; but in one of them the

* Phil. Trans. vol. clxvi. p. 60.

† After the possible influence of hard drying and hardening had suggested itself, I purposely introduced old hay from various localities into the laboratory.

infusion was scumless throughout, while in the other each of the three tubes was heavily laden with scum.

The two chambers containing the alkalized infusion of dried London hay had all their tubes turbid and covered with scum. In the case of the acid infusion of dried hay, the tubes of one of the chambers became turbid, while the tubes of the other chamber remained clear.

The two chambers of alkalized new Heathfield hay-infusion were also in disaccord. In the one chamber all three tubes became turbid and covered with scum, while in the other chamber the three tubes remained sensibly clear and free from scum. Nor did the three tubes of the single chamber charged with the new Heathfield acid infusion present the same appearance; for while one tube became thickly turbid, the other two remained perfectly pellucid.

Amid this confusion, the only point worth dwelling on is, that while no single case of escape occurred with the old-hay infusion, whether acid or neutral, with the infusions of both dried and undried new hay a certain percentage of the tubes remained sterile.

Reflection on these results naturally drew suspicion upon the chambers. They had been used before, and, though carefully cleansed, some unobserved source of infection may have clung to them. This, at all events, seemed the most rational way of accounting for the differences observed between samples of the self-same infusion placed in different chambers. Hence my desire to expose a fresh series of infusions in chambers which had never been used before.

Six new ones were therefore constructed, each of them containing six tubes. These were charged on the 3rd of November with infusions of old London hay, old Heathfield hay, new London hay, and dried London hay. Two chambers were devoted to each infusion, which in the one chamber was neutralized and in the other unneutralized.

The six tubes in each chamber were arranged in two rows of three tubes each. Those nearest to the glass front were called the front tubes, the others the back tubes. The infusion intended for the unneutralized chamber was unboiled before its introduction into the three back tubes, and boiled in those tubes for five minutes afterwards; the infusion for the front tubes was boiled for fifteen minutes before introduction and for five minutes afterwards. These differences in the mode and period of boiling were adopted to ascertain whether they had any influence on the subsequent development of life. In the case of the neutralized chambers, the infusion for the three back tubes was boiled for fifteen minutes outside before neutralization, and five minutes in the chamber after neutralization. The infusion for the three front tubes was boiled fifteen minutes outside after neutralization, and five minutes afterwards in the chamber. If the potash used for neutralization carried germs into the infusion, the difference between five and twenty minutes in the period of boiling might, it was thought, declare itself in the subsequent phenomena.

Four days after its introduction the old Heathfield acid infusion was found turbid throughout and covered with scum. The scum and turbidity were sensibly the same in all the tubes, though the period of boiling varied from five to twenty minutes. On the same day the neutralized infusion of the same hay was perfectly brilliant and free from scum. Three days subsequently, however (that is to say, on the 10th of November), the neutralized tubes also became turbid and covered with scum.

The salient fact here to be noted is, that in neither the neutral nor the acid chamber did a single tube of the old Heathfield hay-infusion maintain its primitive clearness and freedom from scum.

The old London hay behaved substantially as the old Heathfield hay, no single tube escaping either in the neutralized or the unneutralized chamber.

The dried new London hay comes next. A week after its introduction every one of the six tubes containing the acid infusion was turbid and coated with scum. In the neutralized chamber, on the contrary, two only of the back tubes gave way, the third back tube and the three front tubes remaining clear.

On the 3rd of November, moreover, a new chamber of six tubes was charged with an infusion of new London hay. Three of the tubes were neutralized and three unneutralized. Both infusions were introduced into the chamber unboiled, and were boiled afterwards for five minutes. In a week all the tubes had given way, becoming turbid in the same degree and covered to the same extent with scum. The newness of the hay had failed to secure the sterility of the infusions.

Nothing of this kind occurred in the experiments of last year. It was then found that hay-infusions of all kinds were uniformly sterilized by five minutes' boiling.

Guided by such hints as the experiments furnished, I continued to work. On the 4th of November four closed chambers of three tubes each were charged with infusions of old and new Heathfield hay—two chambers with the one, and two chambers with the other. One chamber of each pair contained a neutralized, the other an unneutralized infusion, and the time of boiling was ten minutes. Six days subsequently the infusion of new hay, both neutralized and unneutralized, was found perfectly unchanged. Of the old-hay infusion, on the other hand, only one of the six tubes escaped. The three acid tubes became completely turbid, while two out of the three neutral ones fell into the same condition.

§ 8. *Experiments with soaked Hay.*

Pondering still further on the influence of drying and hardening, and recognizing the necessity of not only wetting but also softening the germs, the thought occurred to me of soaking the hay for some days prior to digesting it. Old London hay was accordingly chopped up and placed in three glass vessels—one containing distilled water, another acidulated water, and a third alkalized water. The superior extractive power of the alkalized liquid was at once manifest; it rapidly assumed a dark colour. The distilled water came next, yielding a colour less deep than that of the alkalized, but more deep than that of the acidulated water. The alkaline and distilled-water infusions emitted a rich odour of hay, while the smell of the acid infusion was very faint, and not like that of hay. The hay was permitted to soak from the 8th to the 11th of November. It was then digested for three hours in the same liquid at a temperature of 120° F., boiled, filtered, and introduced into the closed chambers, where it was reboiled in each case for five minutes.

Prior to digesting the hay in the liquid in which it had been soaked *Bacteria* had developed in swarms. These, of course, were killed by the boiling, and they were not entirely removed by the filtration. The alkaline infusion, indeed, though filtered repeatedly, was sufficiently turbid to prevent the flame of a candle placed behind the tubes containing it from being seen. The same to a less extent was true of the distilled-water infusion. This latter had been divided into two portions, one of which was accurately neutralized, and the other left unneutralized, separate chambers being devoted to each.

From the 11th to the 18th of November the only change observed in any of the infusions was in the direction of increased transparency. They all became clearer with time, the distilled-water infusions becoming particularly clear and brilliant at the top. After two or three days' quiet the alkaline infusion allowed a flame placed behind it to be seen of a deep and brilliant red. The acidulated-water infusion remained entirely unchanged; but this is not worth dwelling on, for in this case, even when exposed to the common air, the infusion resisted infection for a considerable time.

In no case was the fatty scum which had been already so frequently observed formed in any one of the tubes. Some change inimical to the particular organisms which produce this scum must have been caused by the soaking of the hay.

Examined microscopically on the 18th of November these infusions, I thought, exhibited undoubted evidences of Bacterial life. Bacterial forms were unquestionably there in considerable numbers, more particularly in the sediment at the bottoms of the tubes. Nor do I now see any valid grounds for doubting the presence of life; but I was warned against drawing too hastily the conclusion which first prompted itself, by boiling an infusion swarming with active *Bacteria*, and submitting the liquid after cooling to microscopic examination. Here also the dead Bacterial forms were preserved, and it was extremely difficult to distinguish their motions, which were certainly Brownian motions, from those observed in the protected infusions of soaked hay.

The experiment was thought worth repeating. On the 16th of November accordingly chopped bundles of old Heathfield hay and new Heathfield hay, and of old London hay and new London hay, were placed in glass dishes containing distilled water, and were thus soaked until the 18th. They were then moved from the lower laboratory, and taken, with their glass covers, to a distant room at the top of the Royal Institution. Here the four specimens of hay were digested for three hours at a temperature of 120° Fahr. They were filtered, boiled, refiltered, some of them through 100 layers of filter-paper; after which they were introduced into four closed chambers of six tubes each, and then boiled for five minutes.

On the 20th of November the infusions in all the chambers appeared to be as free from organisms as at first. The new Heathfield and the new London hay-infusions in their respective chambers had their somewhat turbid columns surmounted by an exceedingly clear zone of liquid, due, I should consider, to the mechanical subsidence of the particles, had not subsequent experience taught me to regard this appearance as a sign of life.

On the 23rd scum had begun to gather on every tube of the case containing the infusion of old Heathfield hay. On the 30th this scum continued, but there was no trace of it in any of the chambers containing new Heathfield hay, new London hay, and old London hay. These infusions were all somewhat turbid; but the turbidity differed very little from that exhibited when the infusions were prepared.

I spent a good deal of time over these infusions of soaked hay, both with the microscope and otherwise, but the recorded observations would not add materially to our knowledge. I therefore dismiss them with the remark that their general drift was in favour of the idea that the extraordinary resistance to sterilization manifested by the old-hay infusions is the result of hardening and desiccation. The foregoing observations, however, have been noted, more with the view of indicating my line of thought than of claiming for them any value whatever as a demonstration.

§ 9. *Infusions of Fungi.*

Turning from hay to substances in which germs, if they existed, could not be desiccated, I felt pretty sure that infusions of such substances would be unable to resist the boiling temperature. To test the correctness of this view the following experiments were made:—Three different kinds of fungi (red, black, and yellow) were gathered in Heathfield Park on the 13th of October, and digested separately in London on the following day. Three tubes of a closed chamber containing six tubes were charged with the red-fungus-infusion and three with the black, while a second chamber of three tubes was charged with the yellow-fungus infusion. They were all boiled for five minutes after their introduction into the chambers.

For two or three days all the infusions continued clear; but, contrary to my expectation, they subsequently broke down, every tube of the nine becoming turbid with organisms and covered with scum.

Examined microscopically on the 8th of November the red-fungus infusion was found charged with a multitude of spore-like bodies, massed in some places continuously together, in others floating freely in the liquid. Among these ran long filaments, dotted with spore-like specks from beginning to end. There was a considerable number of Vibrios in one of the tubes. The black-fungus infusion contained a mixed population of Vibrios and *Bacteria* with spore-filled filaments. Swarms of *Bacteria* were observed in the red-fungus infusion.

Suspicious of the chambers in which these infusions had been exposed, I had three new ones constructed and provided with new tubes. A fresh supply of fungi was sent to me from Heathfield, a tree fungus being, however, substituted for the black one used in the former experiments. On the 1st of November the three infusions were very carefully introduced into three chambers, a chamber being devoted to each infusion. I thought it advisable to vary the period of subsequent boiling. One tube of the yellow fungus was therefore boiled for five, one for ten, and one for fifteen minutes; but as it was difficult to save the infusion from waste when the boiling was long continued, one tube of each of the other two infusions was boiled for five minutes, and the other two for ten. Tubes charged with the respective infusions were exposed at the same time to the common air.

In two days the outside tubes containing the red- and yellow- fungus infusion became turbid and covered with the fatty scum so prevalent in our laboratory this year. No scum had formed on the surface of the exposed tree-fungus infusion, which, to casual observation, appeared quite black. Closer scrutiny, however, showed that it transmitted the deepest red of the spectrum, and was apparently quite free from floating matter. It changed rapidly during the night of the 3rd, and on the morning of the 4th of November the bottom of this tube was found laden with a heavy dark-brown precipitate, while numerous dark-brown flocculi floated in the liquid overhead, which had become almost as clear and colourless as water. Under the microscope the dark-brown mass resolved itself into confused moss-like patches and long cylindrical sheaths dotted throughout with small dark specks. These filaments with spore-like specks have been of very frequent occurrence in this inquiry.

The deportment of the closed chambers was as follows:—1. Yellow fungus: the liquid in the three tubes remained perfectly and permanently clear and without a trace of the scum which loaded the infusion outside. 2. Red fungus: one of the three tubes became thickly turbid, while the two others maintained their pristine brilliancy. 3. Tree fungus: one of the tubes became thickly turbid, the two others remained permanently clear.

I asked myself why should one tube of the red fungus give way and the others remain intact? The answer seemed at hand. The turbid tube had been boiled for only five minutes, while the clear ones had been boiled for ten. On consulting the adjacent chamber this possible explanation was blown to the winds, for here the turbid tube had been boiled for ten minntes, while its untainted neighbour had been boiled for only five.

Thus, although the more careful repetition of the experiments did not secure every tube from infection, the escape of seven out of nine of them entirely destroys the presumption of spontaneous life development which the first experiments might seem to suggest.

Wishing to observe more attentively the action of common uncleansed air upon boiled fungus-infusions, a tray of 100 tubes was charged with them on the 14th of October. Thirty-five tubes were filled with black, thirty-five with yellow, and thirty with red-fungus infusion. On the 16th of October every one of the yellow-fungus tubes was turbid and covered with a thick, coherent, cobweb-like scum. The surfaces of the black-fungus tubes were also sprinkled with spots of white scum. Turbidity was the only change observed in the red-fungus tubes. They were wholly free from scum.

Examined microscopically on the 2nd of November the yellow-fungus tubes were for the most part found swarming with exceedingly small and active *Bacteria*; the red-fungus tubes also swarmed with *Bacteria*, some beaded Vibrios being mingled with them. In many of the tubes examined galloping monads appeared, attaining an astounding development in the black-fungus infusion. Patches of moss-like matter would appear here and there in the field of the microscope; and it was no uncommon thing to see from ten to twenty monads nestling and quivering in this "moss," and darting actively in and out of it. They put me in mind of frogs amid their spawn; and as I looked at them my belief in the animality of the one was almost as strong as in that of the other. Almost every patch of spawn-like matter had its colony. In some cases hardly any thing but monads was to be seen; but in others the crowding of active Vibrios was so great that the monads wholly retreated from the field.

§ 10. *Infusions of Cucumber, Beetroot, &c.*

The fungi having disappeared on the approach of winter, I turned to cucumber and beetroot, not expecting that their sterilization would offer any difficulty. Two closed chambers were accordingly prepared, left for the proper time in quietness, and on the 7th of November were charged, the one with the cucumber- and the other with the beetroot-infusion. In a few days the infusions in both chambers broke down, first losing their transparency and afterwards loading themselves with fatty scum.

On the 18th of November twenty-four COHN's tubes* were charged with infusions of cucumber, beetroot, parsnep, and turnip, six tubes being devoted to each infusion. They were placed in a vessel of cold water, raised gradually to the boiling-point, and maintained at the boiling temperature for ten minutes. Before their removal from the hot liquid they were one and all plugged with cotton-wool.

On the 30th of November all the infusions were thickly turbid throughout and heavily coated with scum.

From some of the precautions already mentioned it may be inferred that before this point of the inquiry had been reached, I had begun to suspect the atmosphere in which I worked. Hay of various kinds, both old and new, had been exposed and shaken about in the laboratory, the air of which doubtless contained multitudes of spores which diffused and insinuated themselves everywhere. So, at all events, I reasoned. On the 20th of November, therefore, I had infusions of cucumber, beetroot, parsnep, and turnip prepared, far from the laboratory, in one of the highest rooms of the Royal Institution, and introduced into four new chambers of three tubes each. I deemed the precaution of preparing the infusions and introducing them in the distant room sufficient. Accordingly, when the chambers were charged they were carried down, and the infusions boiled in the laboratory.

Two days afterwards the parsnep alone remained clear. This, however, was only a

* See § 4.

respite, for a day or two subsequently it fell into the condition of its neighbours. On the 30th of November both turnip- and parsnep-infusions were turbid throughout, and laden at the surface with thick fatty scum. The cucumber was also heavily laden with scum, which sent long streamer-like filaments into the subjacent liquid. The beetroot agreed with the others in becoming turbid, but differed from them in remaining free from scum. In no case last year did turnip-infusion show the deportment here described. Knowing, then, from multiplied experiments, that turnip possessed no inherent power of life-development, I was forced to refer its present behaviour, and with it the behaviour of cucumber, beetroot, and parsnep, to infection from without.

I once more tried removal to a distant room, with the added precaution of not only introducing the infusions into the chambers upstairs, but of boiling them there. It had been noticed that when the test-tubes were withdrawn from the oil-bath, and the discharge of steam into the chambers ceased, a somewhat violent entrance of the air into the cooling-chamber was the consequence. To sift such air of its germs, both the funnel of the pipette and the open ends of the bent tubes were carefully stopped with cotton-wool. The wool was never removed from the funnel, and it was not removed from the bent tubes until the chamber had thoroughly cooled. The same vegetables were operated on, viz. cucumber, beetroot, turnip, and parsnep. On the 25th of November four chambers were charged with the infusions. On the 30th they were one and all covered with a layer of deeply pitted and corrugated fatty scum. Thus far, then, I was defeated in my efforts to escape contamination.

During these experiments a fact was observed which repeated itself afterwards in other instances. Samples of the different infusions were always exposed to the common air beside their respective chambers, and in general these outside samples became turbid and covered with scum a day or so before the interior tubes gave notice of breaking down; but here, in the case of the turnip, the outside tube continued pellucid and free from life for some time after the inside ones had become turbid with organisms. How could this be? The case of my two trays placed one above the other last year * suggested itself to my memory. In point of life-development it was then found that the lower tray was always in advance of the upper one. As pointed out at the time, the absence of agitation which permitted the germs to sink into its tubes was the cause of the quicker contamination of the lower tray. No other cause appeared to me assignable in the present instance. By some means or other germs had insinuated themselves into my closed chamber, where the tranquillity of the air permitted them to sink into the infusion, and thus produce effects in advance of those produced by the unquiet air outside. So I reasoned.

But how could the germs get into the chamber? I could fix at the moment only upon one way. The weather had changed from warm to cold and from cold to warm. This genial outside temperature sometimes caused the air surrounding the infusions to rise to upwards of 90° Fahr., and we had often to work in this heat. To

* Phil. Trans. vol. clxvi. p. 68.

moderate it, I sometimes partially turned off the gas, thus lowering the temperature of the room 10° or more. The contraction of the air within the closed chambers followed as a matter of course, and the bent tubes being open, I thought the entrance of the external air might be sufficiently rapid to carry germs along with it.

A new chamber of six tubes was therefore prepared upstairs, three of its tubes being charged with cucumber- and three with turnip-infusion on the 27th of November. The pipette funnel and the bent tubes were plugged above with cotton-wool, which was not removed from them afterwards. I took care, moreover, not to alter the gas-stoves in any way. My care was nugatory. In three days every tube of the six was laden with life. Another chamber of six tubes, charged on the 30th of November with cucumber-infusion, and two additional ones prepared on December 1st, shared the same fate.

Slices of cucumber were next digested for three hours; the infusion was filtered, boiled, and such precipitated matter as appeared on boiling was removed by refiltering. The liquid thus prepared was introduced into five thick glass tubes, which were hermetically sealed, placed in a cold oil-bath, gradually heated to 230°, and maintained at that temperature for a quarter of an hour. The tubes being removed and permitted to cool, the infusion was introduced into a chamber of six tubes, and boiled there for five minutes.

The superheating of the infusion did not even retard the development of life, for in less than two days every tube in the chamber swarmed with *Bacteria*. Thus far, then, every attempt at a solution was defeated.

But why, it may be asked, attempt such solutions? Was it mere prejudice against the doctrine of spontaneous generation that prevented me from frankly submitting to the apparent logic of facts, and admitting the experiments just recorded to be a demonstration of the doctrine? By no means. The only prejudice I feel is the wholesome repugnance to accepting momentous conclusions on insufficient grounds. HUME'S celebrated argument has its application here. Taking antecedent experience fully into account, it was easier for me to believe my knowledge imperfect, or my present work erroneous, than to believe the doctrine of spontaneous generation true.

§ 11. *New Experiments on Animal Infusions. Contradictory results.*

In the course of this inquiry I was continually reminded of last year's experiments, when the most complete immunity from Bacterial or fungoid life was so readily secured. I had operated many times with turnip, never finding the least difficulty as to its sterilization. It is certain that the care bestowed in preparing the turnip-infusion on the 20th of November, 1876, was greater than that bestowed upon the same infusion in 1875. But whereas the latter was invariably sterilized by five minutes' boiling, remaining afterwards as pellucid as distilled water, the former, three days after its preparation, became thickly turbid and swarming with life. I extended the present inquiry to other substances whose deportment was familiar to me last year, some of whose infusions, indeed, still remain with me as clear as they were on the day of their preparation.

On the 1st of December, for example, infusions of beef, mutton, pork, herring, haddock, and sole were prepared, and introduced into six closed chambers, each containing three tubes. On the 5th of December the pork, beef, mutton, and haddock were all covered with a fatty corrugated scum. A seventh chamber, containing artichoke-infusion, prepared at the same time, was found on the 5th more turbid than any of the animal infusions, and equally covered with scum. In the animal infusions, indeed, the body of the liquid underneath the scum maintained a surprising brilliancy, the development of life being confined to the layer in immediate contact with the atmospheric oxygen.

On the 5th of December the herring- and sole-infusions were both clear ; but this was only a respite, for on the 6th white spots appeared on the latter, which extended until they covered the whole surface. The herring-infusion remained clear for a week, after which small specks began to appear on its surface. They never reached the development of the scum which coated the other infusions. It sometimes occurred to me that the oil of this fish exercises a certain antiseptic action.

Last year I preserved infusion of herring perfectly pellucid for months, even in a chamber so leaky that the light could be seen through its chinks. I had, moreover, no failure with any of the animal infusions here enumerated. Last year they all remained sweet and clear ; this year, with far greater precaution, I failed to protect any of them from putrefaction. Reflection on these results, renders, I think, but one conclusion possible to the scientific mind. It will be loth to assume that mutton, beef, pork, haddock, herring, and sole had totally changed their natures, and contracted qualities and powers this year which they did not possess a year ago. But if the origination of the observed life be denied to the infusions themselves, there is but one other source to which it could be referred, namely, atmospheric contamination.

It became, indeed, more and more obvious to me that, in consequence of increased virulence in the contagia afloat this year, liberties in the preparation of the infusions or defects in the construction of closed chambers which would have been of no moment a year ago were sufficient to ruin the experiments, and render nugatory the usual means of sterilization. Against such defects I continued to struggle. With a view to stopping all chinks and crannies which might permit of the entrance of contamination, I had some of the chambers carefully coated with oil-silk and others covered with three coatings of strong paint ; and as failure had attended my efforts to procure an uninfected atmosphere upstairs, I had the entire apparatus used for digesting, filtering, and boiling removed to a store-room at the base of the Royal Institution. The floor of the room was of stone, and it was covered by no carpet. Prior to going into it, moreover, I caused my assistant to remove the clothes which he had previously worn in the laboratory and to dress himself in others. The infusions prepared under these conditions were cucumber, melon, turnip, and artichoke, which, from beginning to end, were operated on below stairs. Two chambers were devoted to each infusion, and after the usual boiling in the chambers they were permitted to remain in the store-room throughout the night, being transferred to the warm laboratory next morning.

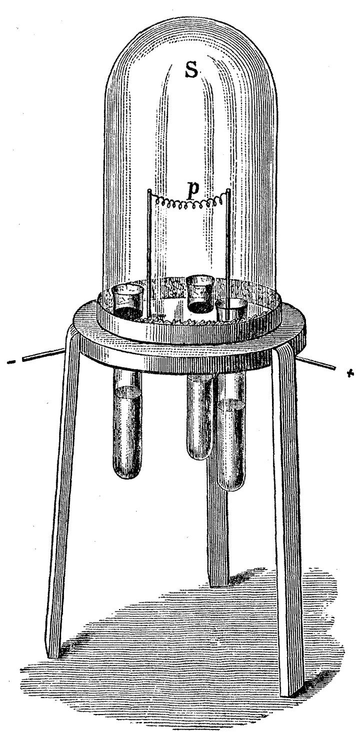
I fully expected that the majority of these chambers would prove sterile. I did not expect to find them all in this condition, because the chambers had been put together in the laboratory, the air of which must have deposited its germs not only on the glycerine-coated interior of the chambers, but also on the inner surfaces of the test-tubes. My expectation, moderate as it was, was not realized. The only noticeable peculiarity in the deportment of the infusions was that they yielded tardily, but in the end every one of them, without exception, broke down.

Was the infection in this case derived from the air of the store-room? I think not; and for this reason:—On the 27th of December four hermetically sealed flasks, charged with a cucumber-infusion which had remained perfectly pellucid for some weeks, were opened in the store-room; four similar flasks, charged with the same infusion, were opened at the same time in the laboratory. On the 31st of December the whole group of the latter four was found invaded by organisms, while those opened in the store-room contracted no infection and developed no life. I do not think, therefore, that the air of the store-room had any thing to do with the contamination of the infusions contained in the closed chambers, but that the contagium already existed in the chambers when they were taken down stairs. They acted as infected houses placed in a salubrious air.

§ 12. *Infusions protected by Glass Shades containing calcined Air.*

I have already described this mode of experiment *. The shades stood upon circular plates of wood, each supported on a tripod (see fig. 6). Under each shade were two upright rods of stout copper wire, and stretching from rod to rod was a spiral (*p*) of platinum wire. The copper wires passed through the slab of wood, their free ends being in the air. The rim of each shade was surrounded by a collar of tin attached by wax to the slab, with a space of about half an inch between the collar and the glass. After the introduction of the infusions and the mounting of the shades, this annular space was packed with cotton-wool. The aim here was to destroy the floating matter of the air by the incandescent platinum spiral. The air heated by the spiral would of course expand, passing outwards through the cotton-wool, while the air reentering, on the cooling of the shade, would be duly sifted by the wool. In my former experiments five minutes' incan descence sufficed to render the air absolutely inoperative on infusions exposed to its action.

Fig. 6.



In the present experiments the period of incandescence was doubled, ten minutes being allowed instead of five,

* Phil. Trans. vol. clxvi. p. 50.

while the wire was raised to the highest possible degree of incandescence. The infusions employed were turnip and cucumber, a group of three tubes being charged with each. After the air had been calcined, the infusions were boiled for five minutes in an oil-bath. With this mode of treatment not a single failure occurred last year, turnip-infusion being among the number of liquids thus treated. This year two days sufficed to render every one of the six tubes turbid with organisms and to cover the infusion with a heavy scum.

I, however, had occasion to doubt the closeness of these shades. The wax intended to seal the junction of the tin collar with the plate of wood had cracked and yielded here and there, and the entry of contamination through such cracks was possible. Six new shades were therefore mounted and surrounded by collars which were imbedded in white lead and firmly screwed down to the plate of wood. The height of the collar, which measured the depth of the filtering layer of wool, was much greater than it had ever been last year. As before, the period of incandescence was ten minutes, during which the platinum spiral was brought as close as possible to its point of fusion.

Each of these six shades covered a group of three test-tubes. Two such groups were charged with turnip, two with cucumber, and two with artichoke-infusion. The infusions, as usual, were boiled for five minutes after calcination. They were all brilliant when prepared; but in two days every one of them had become turbid, and had covered itself with a fatty scum. This gradually augmented until it reached in some of the tubes a thickness of half an inch. The weight of the scum caused it in some cases to bag downwards, forming a kind of inverted cone, the apex of which was more than an inch from its base. These bags finally broke and scattered their organisms in the subjacent liquid.

§ 13. *Further precautions against Infection.*

At the beginning of December, my attention being keenly aroused by those successive failures, I watched more closely than I had previously done the filling of the test-tubes through the pipette. Now and then I noticed minute bubbles of air carried down with the descending infusion. On escaping from the end of the pipette, these small bubbles I concluded would break, and scatter such germs as they contained in the air of the chamber. Last year I should have found it difficult to believe that a cause so small could lie at the root of the observed anomalies; but this year I had learned to respect small causes, and accordingly took measures to effectually exclude the air.

On December 4th three chambers, which had been previously left quiet for several days, were charged with carefully prepared cucumber-infusion, and two other chambers with turnip-infusion prepared with equal care. The following precautions were taken:—The funnel of the pipette formerly employed was broken off from its shank, and for it was substituted a "separation-funnel" with a glass stopcock. This was connected by closely fitting india-rubber tubing with the shank of the pipette. But before the connexion was made, the funnel was filled with the infusion, and the stopcock turned on for a moment, until the liquid issued from the orifice below. The stopcock being then turned off, the flow of the liquid ceased, and the column in the shank below the stopcock was supported by atmospheric pressure. A pinchcock nipped the india-rubber tube at its centre. The portion of tubing above the pinchcock being filled with the infusion, the end of the separation-funnel was introduced into the tube, all air being thus excluded. On turning on the stopcock and releasing the pinchcock, the liquid passed slowly down the shank of the pipette, filling it wholly. The point of the shank was then placed in succession over the test-tubes, the infusion entering them without a single associated bubble. The arrangement was not perfect, but it was an improvement upon previous ones. As before, the charged chambers were placed in a room the air of which was maintained at a temperature of about 90° Fahr.

The result was as follows:—Of the two turnip chambers prepared, as just described, on the 4th, one had completely given way on the 6th. In the other chamber two out of the three tubes had given way, but the third remained permanently brilliant. Previous to this series of experiments I had never succeeded in saving even a single tube of cucumber-infusion; here, however, two out of the three chambers charged with it remained perfectly clear for many days. Subsequently one of these chambers yielded in part, through an accident, but the other chamber is as brilliant at this moment as it was on the day of its preparation many months ago.

Now, as regards inherent power to generate life, the infusion of this chamber was in precisely the same condition as its two neighbours. They, one and all, contained the same infusion; and there is no way of accounting for the observed difference of deportment save by reference to contamination from without. Here we seem to be on the traces of the enemy which has given us so much trouble.

On the 5th of December two additional cases were charged with infusion of melon prepared in the usual way; and on the 12th of December I subjected both these chambers, and those prepared upon the 4th, to a very close scrutiny. The result was instructive. After the introduction of the infusions, and prior to the removal of the separation-funnel, the india-rubber tubing connecting the latter with the shank of the pipette was perfectly closed by the pinchcock. Provided the clasping of the india-rubber tube round the shank of the pipette were perfectly air-tight, the liquid contained in the shank ought to remain there, supported by atmospheric pressure. If, however, the india-rubber tube failed to clasp with sufficient tightness the pipette-shank, air would insinuate itself between the two, and the depression of the liquid would be the consequence. The result observed upon the 12th was this:—In two only of the seven chambers prepared on the 4th and 5th was the liquid column found perfectly supported; and only in these two chambers were the test-tubes, which contained cucumber-infusion, without exception pellucid.

In the five remaining chambers the liquid columns, which had completely filled the pipette-shanks on the 4th and 5th, were found more or less depressed. The tubes in one of the chambers, containing melon-infusion, had become rapidly turbid and covered with scum. The pipette-shank in this case was found entirely emptied of its liquid and filled with air. Another chamber had nine inches, while a third had seven inches of its pipette-shank filled with air. In a fourth chamber only one inch of the pipette-shank was filled with air; here one out of a total of three tubes remained pellucid. Thus, where the closure above was perfect, we had in this instance perfectly pellucid infusions; where it was grossly defective, the infusions gave way in all the tubes; while where the closure was but slightly defective we had the escape of a fraction.

The defects thus revealed came doubtless into play when the infusions were introduced, the descending column of liquid sucking in minute air-bubbles between the india-rubber tubing and the pipette, thus carrying with it the external contagium. Few are aware of the precautions essential to save the experimenter from error in inquiries of this nature. Even with some of our best and most celebrated observers I find no adequate sense of the danger involved in their modes of experimentation.

§ 14. *Experiments in the Royal Gardens, Kew.*

But it was only in exceptional instances, dependent on the state of the air, that even precautions such as those described in the foregoing section secured freedom from contamination. The contagium seemed omnipresent and persistent, and whether it was local or general—due to the accidental condition of our laboratory, or to an epidemic of the air—became a question with me, not by any means to be decided offhand. On this point, then, I held judgment in suspense. The infection was, to all appearance, fully accounted for by reference to the conditions under which I worked; but as regards outbreaks of epidemics the autumn had been a remarkable one, and it seemed well worth investigating whether it was not also a period prolific generally in the germs of putrefaction.

I resolved therefore to break away wholly from the Royal Institution, and, thanks to the friendly permission of the President of the Royal Society, I was enabled to transfer my apparatus to Kew Gardens. By the enlightened munificence of Mr. JODRELL, a new and very complete laboratory had been just erected there, and in it I sought a purer air than I could find at home.

My chambers hitherto had been constructed of wood, but those to be tested at Kew were made of block-tin, and they were carried direct from the tinman's to the gardens without being permitted to come near the infected air of Albemarle Street. At Kew the test-tubes employed were first cleansed with carbolic acid, then washed with a solution of caustic potash, afterwards swept out with distilled water, and finally raised almost to the temperature of redness by a Bunsen-flame. They were then fitted airtight into the chambers with white-lead and tow.

The chambers were closed on the 3rd of January, and allowed to remain quiet until the 8th, when the two most refractory liquids that I had encountered in the laboratory of the Royal Institution were introduced into them. These were infusions of cucumber and melon. There were two chambers devoted to each infusion—four in all; and each chamber embraced three large test-tubes. The period of boiling was that found effectual last year, *i. e.* five minutes. The temperature of the room in which the chambers were placed was maintained, partly by hot-water pipes and partly by a gas-stove, at about 90° Fahr.—a temperature which had been proved eminently favourable to the development of *Bacteria*.

Tubes containing the same infusions were at the same time exposed to the common air of the Jodrell laboratory. These became rapidly turbid and covered with scum. Anxiously inspected during the early days of their trial, the protected tubes showed no signs of giving way. Nor did they yield afterwards. On the 19th of January the four chambers were removed in a van from Kew, and shown in the evening of that day to the members of the Royal Institution, including many Fellows of the Royal Society. The infusions were one and all brilliant, no trace either of turbidity or scum being found associated with any of them. During all my previous efforts (and they had been very numerous) I had never succeeded in saving a single tube of melon-infusion; here, however, every tube of both chambers was intact. The epidemic was thus localized, the obvious cause of it being the contaminated air of our laboratory.

A couple of days subsequent to the removal of the chambers from Kew, a single tube of the cucumber-infusion became turbid, its two neighbours in the same chamber remaining intact. As long as they were kept quiet not one of the other tubes, either of melon or cucumber, gave way. They all remained as pellucid as at first. Their removal from Albemarle Street to the city last year ruined many of our sterilized chambers. I was not therefore prepared to see so little damage done by the transport from Kew.

It may be remarked, in passing, that this infection of an infusion by mere mechanical shaking is an obvious proof that the contagium is not a gas or vapour, but that it consists of particles capable of being detached from the interior surface of the chamber, and endowed with the power of passing into active life.

Two other chambers were exposed at the same time in the Jodrell laboratory, the one containing beef- and the other sole-infusion. They are by no means so sensitive as the cucumber and melon, still one of the three beef-tubes broke down, becoming thickly turbid throughout. Right and left of this tube its two companions remained perfectly transparent. As an illustration of the externality of the contagium, the result was more conclusive than it would have been had all three tubes remained intact; for had the power of developing the organisms which produced the turbidity been inherent in the infusions, its action would not have been confined to a single tube.

It will be understood that when the chamber is lifted from the oil-bath in which its infusions are boiled, the air within the chamber contracts, and an indraught is the consequence. If the entering air be properly sifted, by passing it through cotton-wool plugs, no harm is done; but if it enter an aperture unsifted, it carries its motes along with it. In the beef-chamber just referred to an aperture of this kind, about the size of a pin-hole, was detected. This obviously was the door through which the contagium entered. Through a similar but graver defect in its chamber the sole-infusion also broke down; but in a subsequent experiment with sole-infusion in the Jodrell laboratory, two thirds of the whole number of tubes charged with it remained free from all trace of life.

§ 15. *Experiments on the Roof of the Royal Institution.*

With a view to making nearer home experiments similar to those made at Kew, I had a wooden shed erected on the roof of our laboratory. The shed was provided with benches, water and gas-pipes, and a stove for heating. To an infusion of cucumber, which I had found extremely intractable in the laboratory, my attention was first directed. Two tin chambers of three tubes each were prepared, and transferred to the shed from the workshop where they were made without being permitted to enter our laboratory. The cucumber used for the infusion was also kept clear of the infected air; it was sliced and digested in the shed, the infusion was there filtered, introduced into the tin chambers, and boiled subsequently for five minutes.

The result was not that expected. Not a single tube of either of these two chambers escaped contamination. They one and all behaved like the same infusion in the infected laboratory, becoming in three days turbid throughout and laden with fatty scum.

I have been daily and hourly impressed with the parallelism between these phenomena of putrefaction and those of infectious disease. A further illustration of this parallelism is here presented to us. The clothes of my assistants who prepared the infusion in the shed had been worn in the laboratory, a transfer of infection by one of the modes of transfer known to every physician being the result. The thoughtful physician cannot indeed fail to see the absolute identity of deportment between the contagia with which he is familiar, and those assailants of my infusions against which I have been contending so long.

With regard to the shed my first step, after this preliminary failure, was to disinfect it. This was done by washing every part of it, first with a mixture of carbolic acid and water, and secondly with a solution of caustic potash. When the whole was well dried, new tin chambers furnished with new tubes were introduced. Cucumbers and beef fresh from the market were also digested in the shed, my assistant taking care to cover his legs with clean linen trowsers, and his body with a new blouse. There was one chamber devoted to the cucumber and another to the beef. Into the former the infusion was introduced on the 19th, and into the latter on the 20th of March; each infusion was boiled for five minutes after its introduction.

Let us compare results and draw conclusions. At a distance of eight yards from the shed, viz. in the laboratory, infusions both of beef and cucumber refuse to be sterilized by three hours' boiling. Indeed I have samples of both infusions which have borne five hours' boiling and developed multitudinous life afterwards. But the upshot of this experiment in the disinfected shed is, that every tube of the two chambers, though boiled for only five minutes, contains an infusion which, at the present hour, is as limpid as the purest distilled water.

What shall we say, then? is the infusion in the laboratory endowed with a generative force denied to the same infusion in the shed? Irrespective of the condition of the air, can a linear space of eight yards produce so remarkable a difference? It is only the confusion of mind still prevalent in relation to this subject that renders such a question necessary. Let me add that it suffices simply to wave a bunch of hay in the air of the shed to make it as infective as the laboratory air. Even the unprotected head of my assistant when his body was carefully covered sufficed in some cases to carry the infection.

If any thing were needed to illustrate the extraordinary care necessary on the part of physicians and surgeons, both as regards the clothes they wear and the instruments they use, such illustrations are copiously furnished by the facts brought to light in this inquiry.

§ 16. *Preliminary Experiments on the Resistance-limit of Germs to the temperature of Boiling Water.*

While continuing the conflict and experiencing the defeats recorded in the foregoing pages, a remark of Professor LISTER'S sometimes occurred to me. To apply the antiseptic treatment with success, the surgeon must, he holds, be interpenetrated with the conviction that the germ-theory of putrefaction is true. He must not permit occasional failures to produce scepticism, but, on the contrary, must probe his failures, in the belief that his manipulation, and not the germ-theory, is at fault. This may look like operating under a prejudice; but Professor LISTER'S maxim is nevertheless consistent with sound philosophy and good sense; and if I permitted a bias to influence me in this inquiry, it was one fairly founded on antecedent knowledge, which led me to conclude that the long line of failures above referred to would eventually be traced to my ignorance of the conditions whereby perfect freedom from contamination was to be secured.

I laboured to discover these conditions, and to learn something more regarding the nature of the contamination—its origin, persistence, and manner of action. When these researches began, five minutes' boiling, as I have frequently stated, sufficed to sterilize the most diversified infusions. Here we have frequently extended the time of boiling to ten and fifteen minutes, and, in some cases glanced at above, to immensely longer periods, without producing this result. I desired more exact knowledge as to the limit of endurance, and with this view, on the 22nd of December, had six bulbs charged with an infusion of cucumber, sp. gr. 1004. They were then plugged with cotton-wool, hermetically sealed, and subjected to the boiling temperature for 10 minutes. Six other bulbs, charged with the same infusion and treated in the same way, were boiled for 30 minutes. Finally eight bulbs, similarly charged, were boiled for 120 minutes.

On the 23rd of December three of the first group of bulbs, three of the second, and five of the third, having their sealed ends filed off, were exposed to a tolerably constant temperature of about 90° Fahr. Not one of these twenty bulbs preserved itself free from life. On the 25th of December every one of them had given way to cloudiness and turbidity.

There was, however, a marked difference between the sealed and the unsealed bulbs.

To the latter, it will be remembered, the air had access through the plug of cotton-wool, while to the former no air had access, save the small quantity imprisoned above the infusion when the necks of the bulbs were sealed. The aerated bulbs grew rapidly and thickly turbid, while a passing cloudiness was all that showed itself in the sealed ones. This soon disappeared, and left the infusions apparently intact. In fact it required some attention to detect the appearance of this fugitive life, which existed only so long as there was oxygen to sustain it. I have ranged the sealed and unsealed tubes side by side in groups. To the most cursory observation the difference between them is obvious. The experiment strikingly illustrates the dependence of the special organisms here implicated on the oxygen of the air.

The experiments were pushed still further on the 28th of December. Two bulbs of cucumber, two of melon, two of turnip, and two of artichoke were then plugged, sealed, and maintained at the boiling temperature for four hours. Six of the eight bulbs burst in the operation, but two of them, a bulb of melon and one of cucumber, bore the ordeal uninjured. After cooling, their sealed ends being broken off, they were placed in the warm room. The melon remained permanently sterile, but in two days the cucumber-infusion became turbid and laden with fatty scum.

Eight similar bulbs were boiled on the same day for five hours and a half. Four of them burst, but four remained intact. Of these, two contained cucumber-, one melon-, and one turnip-infusion. Three out of the four bulbs were sterilized by the long-continued boiling, but one cucumber-bulb passed through the ordeal unscathed. Two days after the operation it swarmed with life, and was covered with a fatty scum formed of matted *Bacteria*.

Many similar experiments were subsequently made. On the 27th of January, for example, six bulbs of turnip-infusion were boiled for 220 minutes, six for 300 minutes, and two for 305 minutes. Suspended in the air above each infusion was a sprig of old Colchester hay, this being purposely introduced to augment the chance of infection. Notwithstanding its presence the bulbs were one and all permanently sterilized. The specific gravity of the infusion was in all cases 1007.

The sprigs of old hay were afterwards shaken into the liquid, but they produced no effect. For weeks afterwards the infusion remained clear. Was this impotence to generate life due to the fact that the nutritive power of the infusion had been destroyed by the "blighting influence of heat?" Not so; for when the same infusion was infected by a sprig of fresh hay, by a small pellet of cotton-wool rubbed against the dusty shelves of the warm room, or by a speck of another infusion containing *Bacteria*, it never failed to develop life. The only observed difference between the effect produced by the dry hay or dust and the living *Bacteria* was purely a difference of time. Inoculation with the finished organisms acted more rapidly than infection with the dust, but the effects were the same in the end.

On the 27th of January also nine melon-bulbs were treated exactly like the turnip, being furnished with sprigs of old Colchester hay, plugged with cotton-wool, and hermetically sealed above the plugs. Six of them were boiled for 215 minutes, and three for 220 minutes. They were one and all permanently sterilized; but, like the turnip, all of them were open to infection by fresh hay, dry dust, or living *Bacteria*. The specific gravity of the melon-infusion was 1008.

§ 17. *Further Experiments on the Resistance-limit of Germs to the Boiling Temperature.*

The amount of boiling which turnip-infusion failed to withstand is shown by some of the foregoing experiments; but to determine the limit of its resistance we must begin with shorter periods. On the 1st of March, therefore, eight groups of pipette-bulbs were charged with turnip-infusion which had been prepared in an atmosphere purposely infected with the germs of old Heathfield hay. In every case, moreover, a sprig of the same hay was placed in the air above the infusion. The bulbs had their necks plugged with cotton-wool, and were hermetically sealed above the plug. In this condition the respective groups were boiled for the following times:—

1st group	15 minutes.
2nd ,, ...	30 ,,
3rd ,, ...	45 ,,
4th ,, ...	60 ,,
5th ,, ...	75 ,,
6th ,, ...	90 ,,
7th ,, ...	105 ,,
8th ,, ...	120 ,,

After boiling they were removed, permitted to cool, had their necks broken off by a file, and were afterwards exposed to the temperature of our warm room. It may be remarked that the infusion gradually deepened in colour from the 15-minute period, where the colouring was hardly sensible, to the 2-hour period, where the colouring became deep yellow. The effect was doubtless due to the oxidation of the infusion, which, notwithstanding the colour, was in all cases highly transparent.

Two days after their preparation, every tube of the series had become turbid and had begun to cover itself with scum.

On March the 6th the periods of boiling were prolonged with a fresh infusion. Two groups of tubes were, on that day, exposed to boiling water for the following times:—

1st group	180 minutes.
2nd ,, ...	240 ,,

On the 8th of March all the members of the first group were turbid and covered with scum. The second group was completely sterilized. This latter result is quite in accordance with the experiments made on the 27th of January. Turnip-infusion was then boiled for periods varying from 220 minutes to 305 minutes, complete sterilization being in all cases the consequence. These results were subsequently checked by a continuous series of experiments extending over periods of boiling varying from one to six hours. Up to three hours the infusion resisted sterilization; but when the periods of boiling were prolonged to four, five, and six hours respectively, all the bulbs became permanently barren. The liquid continued in the highest degree transparent, and in colour a brilliant orange-brown.

Experiments intended to determine the limit of resistance of cucumber-infusion were made on the 24th of February. Nine pipette-bulbs were then charged, plugged, hermetically sealed, and subjected to the boiling temperature for the following times:—

1st bulb	15	minutes.
2nd "	30	"
3rd "	45	"
4th "	60	"
5th "	120	"
6th "	180	"
7th "	240	"
8th "	300	"
9th "	360	"

After boiling and cooling they had as usual their ends broken off by a file. The result here was that at the 5th bulb, which corresponded to a boiling for two hours, the life-development suddenly ceased. All the tubes boiled from three to six hours inclusive were completely sterilized.

The infusion in this case had been diluted by an accident, so that its specific gravity was hardly above that of distilled water. On the 28th of February, therefore, a fresh infusion having a specific gravity of 1006 was prepared, and introduced into a series of bulbs exactly as in last experiment. The bulbs were exposed to the boiling temperature for the following times:—

1st bulb	15	minutes.
2nd "	30	"
3rd "	45	"
4th "	60	"
5th "	120	"
6th "	180	"
7th "	240	"
8th "	300	"
9th "	360	"

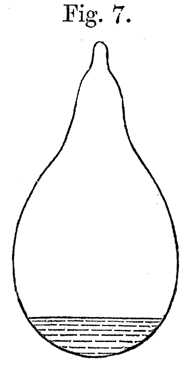
The result here was that at the 6th bulb, which corresponded to three hours' boiling, the life-development suddenly ceased. All the bulbs boiled from 15 minutes to 180 minutes inclusive proved fruitful; while from 240 minutes to 360 inclusive all were completely sterilized. As in the case of the turnip-infusion, the cucumber subjected to long periods of boilihg assumed an orange-brown tinge.

Comparing these results with those obtained with the turnip-infusion, it will be observed that cucumber and turnip exhibit about the same resistant power: three hours' boiling, and less, failed to sterilize both of them; four hours' boiling, and more, rendered both of them permanently barren.

The cucumber-infusions prepared on the 22nd and 28th of February were connected with the atmosphere through the cotton-wool plugs; but no attempt had been made to remove its floating matter from the air above the infusions. On the 22nd, however, four bulbs of the infusion were also prepared, charged with filtered air, left unplugged, and hermetically sealed. The same was done with four bulbs on the 28th of February. Each group was subjected to periods of boiling of 15, 30, 45, and 60 minutes respectively. All of them became turbid; but it was interesting to notice the gradual and obvious fall of life from the 15-minute to the 60-minute period. Could the *Bacteria* have been counted, and the result graphically represented, the ordinate corresponding to the abscissa 15 would have been found very considerably longer than that corresponding to the abscissa 60.

The method of experiment here for the most part pursued was employed by SPALLANZANI and NEEDHAM. It was afterwards extensively applied by the late excellent Professor WYMAN, of Harvard College, while in 1874 it was materially refined and improved upon by Dr. WILLIAM ROBERTS of Manchester. The method is hampered by one grave doubt. The air, plus its floating matter, is imprisoned in the sealed bulbs, so that the heat applied has not only to destroy the germs clasped by the infusion, but also those diffused through the supernatant atmosphere. Now it is not certain whether an amount of heat which would be absolutely destructive to a germ embraced by a hot liquid may not be wholly ineffectual when acting on a germ floating in vapour or air. Throughout SPALLANZANI'S and NEEDHAM'S experiments, throughout those of WYMAN and ROBERTS, and throughout my own, as reported in this section and the last, this possibility of error runs. Such experiments, in short, do not enable us to state with certainty the temperature at which an infusion is sterilized, because the germs which most pertinaciously oppose sterilization may not belong to the infusion at all, but to the adjacent air.

Fig. 7.



The most astonishing cases of resistance to sterilization observed by WYMAN were associated with this particular mode of experiment. The possible action of the uncleansed air, moreover, was in his case augmented by the fact that he employed quantities of liquid, very small in comparison with the size of his flasks. In some of his earlier experiments the volume of air was more than thirty times that of the infusion. These relative volumes are represented in the annexed figure (fig. 7), copied from WYMAN'S Memoir of 1862*.

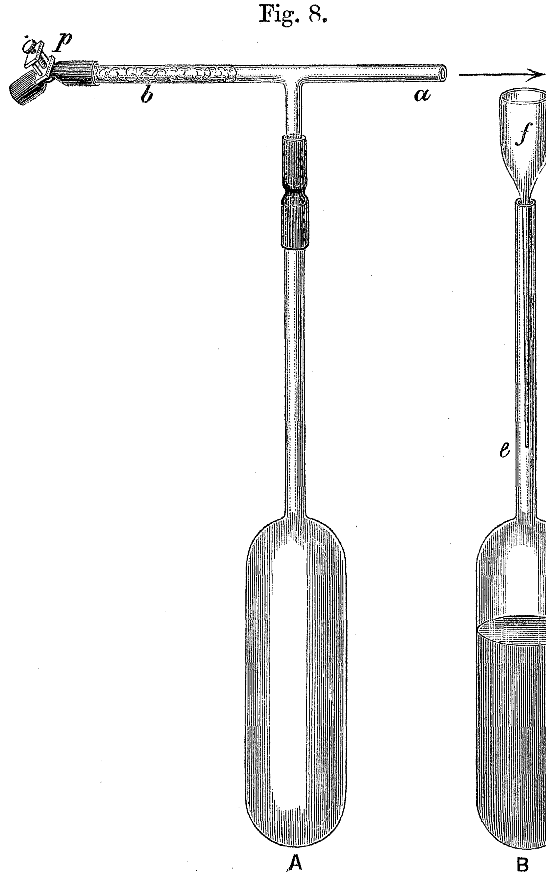
* SILLIMAN'S American Journal, vol. xxiv. p. 80.

§ 18. *Change of Apparatus. New Experiments with Filtered Air.*

The source of possible error referred to in the last section had been long present to my mind, and I had already taken measures to avoid it. On the 2nd of January, 1877, an infusion of turnip (sp. gr. 1006) and an infusion of melon (sp. gr. 1008) were prepared and introduced into a series of pipette-bulbs in the following manner:—One end *a*, fig. 8, of a glass T-tube was connected with an air-pump, the other end *b* was closely plugged with cotton-wool, while to the third branch of the T-tube the neck of the pipette-bulb A was attached by india-rubber tubing. A piece of the same tubing, furnished with a pinchcock *p*, was also attached to the free end of the T-tube beyond the cotton-wool.

The bulb A was exhausted three times in succession, the pinchcock *p* being closed, and was three times filled with filtered air, the pinchcock being opened. At the third exhaustion the bulb was raised to a very high temperature by a Bunsen flame, and finally filled with filtered air. It was then plunged for a minute into ice-cold water, from which it was afterwards removed, detached from the T-tube, and then charged with the infusion by means of a narrow pipette, *f e*, shown at the top of B, fig. 8.

Fig. 8.



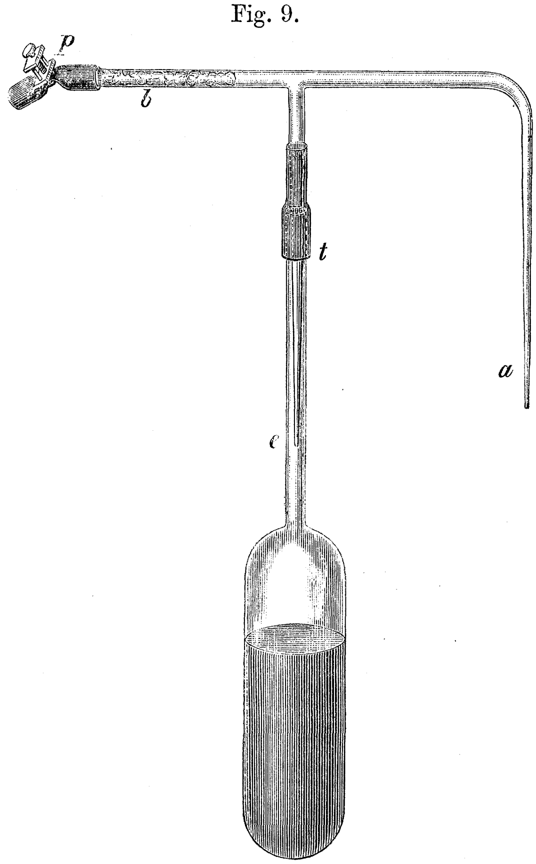
The rationale of the above proceeding is this:—On quitting the ice-cold water for the warmer air of the laboratory, expansion of the air within the bulb would occur. This would cause a gentle motion from within outwards, opposing all indraught of contaminated air. The entry of the infusion into the bulb would, I thought, also promote this outward motion. On the removal of the pipette, which occupied but a very small portion of the neck of the bulb, a little warmth was applied to the latter, and during its application the neck was plugged with cotton-wool. The air entering through this plug to supply the place of the small quantity displaced by the warmth would, I concluded, reach the interior of the bulb perfectly sifted of its floating matter. The necks of the bulbs were hermetically sealed, and the infusions maintained for ten minutes at the temperature of boiling water. After a lapse of twelve hours their sealed ends were broken off by means of a file.

In our experiments on the 28th of December turnip and melon subjected to ten minutes' boiling entirely gave way. In these present experiments, where greater care was taken, two out of the six turnip-bulbs and three out of the six melon-bulbs remained permanently barren. Even this amount of success proved afterwards so exceptional that it might be fairly regarded as accidental.

On the 4th of January the experiments were continued. The pipette-bulbs employed were first carefully washed with carbolic acid, which was removed as far as possible with ordinary water. They were then washed with a solution of caustic potash, and finally rinsed out with distilled water. They were not subjected to the action of the Bunsen flame. The infusions employed were turnip (sp. gr. 1006) and melon (sp. gr. 1008), eight bulbs being filled with each infusion.

I could not be certain that the motion of the liquid fillet at the end *e* of the pipette with which the bulb B, fig. 8, had been charged, had not drawn into the neck of the bulb a modicum of the external air. In the present experiments, therefore, the method of charging the bulbs was modified in the following way:—The glass T-tube employed in our last experiments had its end *a*, which was to be connected with the air-pump, drawn out to a small orifice and bent as in fig. 9. The branch connected with the bulb was also drawn out to a tube of fine bore, which entered the neck of the bulb for some distance to *e*, the thicker part above being connected with the neck of the bulb by india-rubber tubing. The end *b*, as before, was plugged with cotton-wool and provided with a pinchcock, *p*. The object here aimed at was that the liquid should be discharged into the bulb far below the india-rubber connecting-piece, and that during the discharge it should pass only through filtered air.

Fig. 9.



Each bulb was exhausted in the manner already described, and refilled three times in succession. When last filled it was plunged for a minute or so into iced water, with the view of rendering the air within the bulb denser than that without. The pinchcock *p* being closed, the whole apparatus was then detached from the air-pump. On being lifted from the iced water into the warmer air there was a gentle outflow of air from *a*.

The mode of charging the bulb was this:—The point *a* was well sunk into the infusion, and the associated bulb, B, was plunged into boiling water. There was an immediate outrush of air from a which bubbled through the liquid. As soon as the bubbling had relaxed a little, a being still submerged, the bulb was transferred to iced water. A shrinking of the warm air was the consequence, and through a the infusion was forced by atmospheric pressure. It descended the middle branch of the T-tube, and was discharged from its end e into the bulb. The quantity of liquid obtained by a first immersion in the iced water was not sufficient to charge the bulb; but by repeating the process of heating and chilling two or three times, the point a never being permitted to quit the infusion, any required quantity was with ease and accuracy introduced. The neck of the bulb was finally detached from the T-piece by loosening the india-rubber tube t. The bulb was then slightly warmed so as to cause an outflow from within, and while this outflow continued the neck was plugged with cotton-wool. It was sealed above the plug, and after the cooling of the infusion the sealed end was broken off with a file.

It is not my intention to take up the Society's time in describing in detail the numerous experiments made in accordance with this method, or the variety of infusions employed in testing its efficacy. Suffice it to say that, notwithstanding all my care, the results were chequered throughout. Sometimes success would seem complete, but a repetition of the experiments—and I never felt safe without frequent and varied repetition—would, as before, present the success in the light of an accident. I am, however, secure in stating that by pursuing this plan I have in some cases effected complete sterilization by an amount of boiling which, twenty times multiplied, has failed to produce this effect when less accurate methods were resorted to. I have for example placed side by side in my collection a series of organic infusions as pellucid as distilled water which have been rendered permanently sterile by an exposure to the boiling temperature for five and ten minutes respectively, and a second series containing the same infusions boiled for 30, 120, and 330 minutes respectively, and which nevertheless are muddy throughout and covered with scum.

Weeks of labour have been devoted to these experiments, nor did they exhaust the trials actually made. Another mode of proceeding was this. Pipette-bulbs were prepared by having a portion of their necks drawn out to a tube of very fine bore. The open end being connected with an air-pump, the bulb was exhausted and filled with filtered air several times in succession. In the final experiment the bulb was charged with one third of an atmosphere of cleansed air; and while this pressure was maintained by the air-pump the narrow tube was hermetically sealed. Each bulb was afterwards heated almost to redness in the flame of a Bunsen lamp. It was charged by inverting the bulb, dipping the sealed end into the infusion, and breaking it off underneath the surface. The liquid entered until the bulb was two thirds filled, when the narrow tube was again sealed without permitting its open end to quit the infusion. A great number of experiments were thus executed, the results of which distinctly favoured the conclusion, though they did not to my satisfaction prove it, that the resistant germs were not to be wholly ascribed to the air, but that they had survived in the liquid.

§ 19. *Final proof that the Resistant Germs are embraced by the Infusion. Examples of Resistance both in Acid and Neutral Liquids.*

I have here touched upon the question which chiefly harassed me at the time to which I now refer. It was this:—Have the germs, which under the circumstances here described produced life, been really embraced by the infusion itself during the time of heating? The liquid, it will be remembered, had to pass through the neck of the bulb, and it could not descend from the neck into the bulb without leaving a film adherent to the internal surface of the neck. This film, I reflected, might dry in part by evaporation; it might, in doing so, leave germs behind which would be very differently circumstanced from those in the liquid. To germs thus exposed, not to the heat of water, but to the possibly less effective heat of vapour and air, the observed life might I thought be due. Before closing definitely with the proposition that the surviving germs had actually been in the liquid, the possibility to which I have just referred had to be shut out.

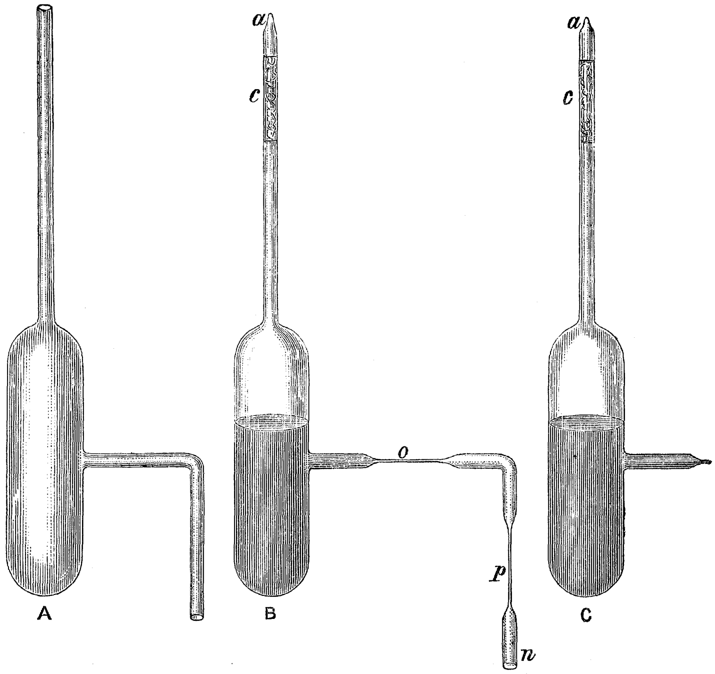
The evil was to some extent mitigated by a suggestion of my assistant to charge the bulb, not through its own neck, but through a narrow tube issuing at right angles to the neck. But even here a portion of the neck and of the higher interior surface of the bulb was trickled over by the infusion. The difficulty was finally met by causing the lateral tube to issue from the centre of the bulb itself, and forcing the infusion into the bulb by atmospheric pressure, until the surface of the liquid stood clearly above the lateral orifice. To this level the liquid rose without wetting any portion of the surface against which it did not permanently rest.

The precise method pursued in preparing and charging the bulbs was this:—First, the bulb as sent to us by the glass-blower is represented at A, fig. 10. Its neck is first plugged with cotton-wool (c) and hermetically sealed as at B, fig. 10. The lateral tube is then drawn out to almost capillary narrowness at o and p. The end n is connected with an air-pump, by which the bulb is exhausted, and after two or three emptyings and fillings, it is finally charged with one third of an atmosphere of thoroughly filtered air. While the pump attached to n maintains this pressure within the bulb, the capillary tube p is sealed with a lamp. The bulb and its appendages are then heated nearly to redness in a Bunsen flame, all life adherent to the interior surface being thus destroyed.

The end p is then introduced into the infusion, pressed against the bottom of the vessel that contains it, and thus broken. The external pressure of a whole atmosphere, having but one third of an atmosphere within the bulb to oppose it, forces the liquid through the lateral tube. It enters the bulb, gradually rising until it reaches the orifice, and rises above it. When the pressure within is exactly equal to the pressure without, two thirds of the bulb are occupied by the liquid.

The infusion then extends without breach of continuity from the bulb B to the vessel in which the end p is immersed, the uncleansed air being thus completely excluded. A small gas-flame is carefully applied at o. The liquid within the narrow tube vaporizes, and the vapour drives the liquid to some distance right and left from the place of

Fig. 10.



heating. In the absence of the liquid the fine tube reddens, fuses, and is hermetically sealed. The aspect of the bulb after it has been thus charged is shown at C, fig. 10.

By this method, on the 20th of February sixteen bulbs were charged with infusions of old Heathfield hay and of a hard wiry hay from Guildford, not old. They were divided into four groups, four bulbs in a group. Each group embraced two acid and two neutral infusions. They were boiled for the following times:—

1st group	10 minutes.
2nd ,, ...	20 ,,
3rd ,, ...	30 ,,
4th ,, ...	60 ,,

After the bulbs had sufficiently cooled, their sealed ends were removed by a file.

On the 21st of February, less than twenty-four hours after their preparation, all these bulbs showed signs of yielding. On the 22nd they were all turbid, while, as regards the comparison of acid and neutral infusions, their condition was this:—

1st group.	10 minutes.	Guildford neutral distinctly more turbid than Guildford acid. Scum on former, none on latter. Old Heathfield neutral not to be distinguished from old Heathfield acid. Both turbid and covered with scum. Much lightened in colour.
2nd group.	20 minutes.	Guildford neutral distinctly more turbid than the acid liquid. Old Heathfield neutral more turbid than the acid infusion.

3rd group.	30 minutes.	Guildford neutral a little more turbid than Guildford acid: difference small. Old Heathfield neutral more turbid than the acid infusion; the former with scum, the latter none.
4th group.	60 minutes.	Guildford neutral distinctly more turbid than Guildford acid; the former with scum, the latter free from scum. Old Heathfield neutral somewhat more turbid and scummy than the acid liquid: difference not great.

Here I take it to be absolutely certain that the germs which resisted sterilization were contained in the liquid. The maximum period of boiling was 60 minutes.

On the 22nd of February four groups of bulbs were charged as above described with the same two infusions, and the periods of boiling prolonged as follows:—

1st group	90 minutes.
2nd "	120 "
3rd "	180 "
4th "	240 "

As in the last case, neutral and acid infusions of each kind of hay were operated on. This was the result:—On the evening of the 23rd, that is to say, 24 hours after their preparation, every one of these bulbs disclosed to the practised eye that organisms existed within it. At 2 P.M. on the 24th they all swarmed with life. The old Heathfield bulbs, both acid and neutral, were turbid and covered with scum, the very weakly-acid infusions being indistinguishable in appearance from the neutral ones. In the case of the Guildford infusion, however, the scum on the neutral infusions was richer and heavier than that on the acid ones.

Four hours mark the limit to which the boiling was carried in these experiments. On the 27th of February the periods of boiling were still further prolonged. Four groups of bulbs charged with infusions of the same two kinds of hay, both acid and neutral, were on that day boiled for the following times:—

1st group	300 minutes.
2nd "	360 "
3rd "	420 "
4th "	480 "

I had previously boiled infusions of old Heathfield, old London, and old Colchester hay for 5 hours, and found them afterwards permanently barren. In the present instance, also, all the bulbs boiled for 5 hours, 6 hours, and 7 hours were completely sterilized. They remained ever afterwards perfectly brilliant. This, with one exception, was also the deportment of the group of bulbs boiled for 8 hours. The exception was a neutralized bulb of Guildford infusion, which became turbid and covered with scum. Considering the severity with which the bulb had been treated prior to charging, and considering the mode of charging it, I do not think that the life developed could have been the product of external germs. Through profound and thorough hardening and desiccation, through defect of contact with the liquid or some other cause, some germs in the infusion itself had, I doubt not, been enabled to withstand the extraordinary ordeal here described.

Was it "the blighting influence of heat" that deprived these 8-hour bulbs of the power of spontaneous generation? Whatever be the meaning attached to such language the reply is obvious, that the "blighting" was the same for all the bulbs; and yet we find one of them, which, when taken from the boiling water, was perfectly brilliant, rendered in two days muddy with organisms. Further, it was only necessary to wash with perfectly sterilized distilled water the adherent germs from a small bunch of hay, and to inoculate the clear infusion in an 8-hour bulb with the washing-water, to cause it within four and twenty hours to become turbid throughout. To speak more definitely, 14 hours in the warm room were found sufficient to cloud the infected 8-hour bulb with *Bacteria.* Thus the infusion, when living germs are restored to it, shows its perfect competence to develop them, and it was solely the destruction of the germs which it possessed before it was boiled that rendered it sterile afterwards.

It is worth bearing in mind that the particular kinds of hay whose germs manifested these extraordinary powers of resistance were so sapless and indurated that the specific gravity of their infusions, even after five hours' digesting, could not without difficulty be sensibly raised above that of distilled water. This was more especially the case with old Heathfield and old Colchester hay, the infusions of which, though highly coloured, were marked, almost without exception, "specific gravity 1000." Old London hay-infusion was usually 1003, while infusions of new Heathfield hay were raised without difficulty to 1007*.

As already stated, I never felt safe in these experiments until they were checked by careful repetition. A partial corroboration has been already adverted to. But on the 2nd of March I had the same infusions prepared, kept in a cool place throughout the night, and introduced into four groups of bulbs next morning. The infusions were all brilliant. During the process of boiling one group of bulbs blew up, hence one link is absent from the series. I thought the 4-hour group could be best spared, and therefore selected it for omission. We had thus the infusions subjected for one, two, three, five, and six hours respectively to the boiling temperature. The three first hours agree exactly with their predecessors. No single bulb within these limits was sterilized, all of them became turbid throughout and loaded with scum. One bulb of the 5-hour group and one of the 6-hour group also became turbid and flocculent within, but without any scum upon the surface. As in the case of the 8-hour bulb already mentioned, this appearance

* A comparative experiment on dried and undried peas, described in the 'Proceedings' of the Royal Society (1877, vol. xxv. p. 503), may be referred to here as illustrating the manner in which desiccation restricts diffusion, and thus tends to preserve the integrity of the desiccated germ. I suppose the original mineral salts of the hay were still retained in the old samples; but hot water appeared to have little power of extracting them. The resistance of the hay in this respect appeared to be shared by its germs.

of life was, I doubt not, due to stray germs of exceptional resisting power, which maintained themselves unscathed in the infusions after their fellows had been destroyed.

By multiplied experiments of a similar character executed subsequently, and fortified by others made in a different way, all doubts as to the real ordeal to which the germs had been exposed were set at rest. A flood of light, moreover, was thrown upon the difficulties recorded in the foregoing pages. Prior to the introduction into our laboratory of the particular samples of desiccated hay whose adherent germs had manifested such extraordinary powers of resistance, infusions of all kinds, even those of hay itself, were sterilized with ease and certainty. But the old London, the old Heathfield, the Guildford, and the old Colchester hay brought a plague into our atmosphere, and thus the infusions of other substances, some samples of hay included, became the victims of a pest entirely foreign to themselves. The failure to sterilize cucumber, turnip, beetroot, artichoke, melon, beef, mutton, haddock, herring, sole, was plainly due to the fact that their infusions had been prepared in an atmosphere, or brought into contact with vessels, contaminated with germs which have been here shown capable of resisting 240 minutes' boiling. It is obvious from all this that to speak of an infusion being rendered barren by such or such a temperature, is simply to use words without definite meaning; because the temperature at which any infusion is sterilized depends upon the character and condition of the germs which find access to it. The death-temperature, for example, may be more than three hours in London and less than three minutes at Kew*.

I may cite here two conspicuous illustrations of the infective energy of those desiccated hay-germs in two infusions which, under ordinary atmospheric conditions, are very easily sterilized. On the 30th of March five pipette-bulbs were charged with clear beef-infusion and boiled for the following times:—

1st bulb	60	minutes.
2nd ,,	120	,,
3rd ,,	180	,,
4th ,,	240	,,
5th ,,	300	,,

After cooling, the sealed ends were broken off, the air being admitted through cotton-wool plugs. Every one of these bulbs became charged with organisms. In the shed, eight yards off, this beef-infusion was, as already reported, sterilized by five minutes' boiling.

Precisely the same experiment was made on March 30 with pellucid mutton-infusion. Not one of the bulbs was sterilized. All of them are at this moment charged with life.

* I have already described the distribution of *Bacteria*-germs in the air as "Bacterial clouds." Were our vision sufficiently sharpened to see the manner in which such germs are distributed over the surface of a meadow, we should not, I am persuaded, find that distribution uniform. We should, in my opinion, find the germs grouped in crowds, with comparatively free interspaces, like violets on an alp, or mushrooms in a field. It is therefore conceivable that two bunches of hay from the same meadow may differ from each other in deportment.

It behoves those engaged in the industry of preserved meat and vegetables to keep clear of the old-hay contamination. Probably they from time to time have encountered difficulties and disappointments which they could not explain, but which may be solved by reference to the results here set forth. But above all the practical question arises:—May not the surgeon have to fight sometimes against enemies like those here described? The particular germs with which I have been so long contending cause, as we have seen, both fish and flesh to putrify. How would they behave in the wards of an hospital? Can they cause wounds to putrify? and if so, would they succumb to the disinfectants usually applied? These are questions the weighty import of which will be best understood by the enlightened follower of the antiseptic system, and which he will know how to answer for himself.

§ 20. *Remarks on Acid, Neutral, and Alkaline Infusions.*

In the foregoing section reference was made to the comparative deportment of acid and neutral infusions. There can be no doubt of the fact that, for the nutrition and multiplication of *Bacteria*, acid infusions are less suitable than neutral or slightly alkaline ones. In acid infusions exposure to the common air sometimes copiously develops *Penicillium*, while it fails to develop *Bacteria*. It is also true that exposure for a certain time to a certain temperature may in many cases prevent the appearance of life in an acid infusion, and fail to prevent it in a neutral or slightly alkaline one.

In the present inquiry this has been frequently found to be the case. I have many closed chambers, for example, to which the process of "discontinuous heating," to be subsequently described, has been applied; and with them it has proved a common experience that an amount of heating which has rendered acid infusions of hay permanently barren has failed to sterilize the corresponding neutral infusions. Moreover, in the cases just recorded, where single bulbs escaped sterilization though exposed for five, six, and eight hours to the boiling temperature, it was always a neutral bulb that kindled into life. To these instances another may be added here. On the 22nd of March an infusion of the wiry Guildford hay already referred to was divided into two parts, one of which was neutralized and the other left acid. Five pipette-bulbs were filled with the one infusion and five with the other. After hermetic sealing they were all completely submerged in water and boiled for six hours. Every one of the acid bulbs was sterilized by this process, while in two days three of the five neutral ones became turbid and covered with scum.

The best thought that I have been able to bestow upon this subject does not induce me to lean towards the explanation suggested by M. Pasteur, namely, that the germs escape the destructive action of the heat because they are not wetted by the alkaline or neutral liquid. From the comparative action of alkalized and acidulated water upon hay, I should be inclined to infer that the wetting of its germs by the former would be more prompt than by the latter. The question, I think, is not one of wetting, but of relative nutritive power. Two *Bacteria*-germs of equal vital vigour dropping from the atmosphere, the one into a neutral or slightly alkaline, the other into an acid infusion, soon cease to be equal in vigour. The life of the one is promoted, the life of the other only tolerated by its environment. When the temperature surrounding both is raised to a prejudicial height the one will suffer more than the other, because equally inclement conditions are brought to bear upon constitutions of different strengths; and if the temperature be sufficiently exalted or sufficiently prolonged to become fatal, the more weakly organism will be the first to give way. A germ, moreover, brought close to the death-point in a neutral or an alkaline infusion may revive, while in an acid one it may perish—just as proper nutriment may rescue a dying man while improper nutriment would fail to do so. These elementary considerations, founded on the demonstrable fact that *Bacteria*-germs are more fully vivified and better nourished in neutral infusions than in acid ones, suffice, I think, to explain the observed difference of action. At all events, these are the thoughts which have become rooted in my mind, through long observation and long pondering of this question*.

§ 21. *Remarks on the Germs of* Bacteria *as distinguished from* Bacteria *themselves.*

The failure to distinguish between these stubborn germs and the soft and sensitive organisms which spring from them has been a fruitful source of error in writings on Biogenesis. In his able and important paper, "On the Origin and Distribution of *Bacteria* in Water, and the circumstances which determine their existence in the Tissues and Liquids of the Living Body," Dr. BURDON SANDERSON, for example, has described experiments from which, in my opinion, very incorrect conclusions have been drawn. He exposed to the common air vessels containing PASTEUR'S solution, which when inoculated with fully developed *Bacteria* enables them freely and copiously to increase and multiply; he even caused the air to bubble through the solution, and finding that though *Torula* and *Penicillium* were luxuriantly developed in the liquid, *Bacteria* never made their appearance, he concluded, "not merely that the conditions of origin and growth of *Bacteria* and fungi are considerably different, but that, as regards the former, the germinal matter from which they spring *does not exist in ordinary air*"†. These italics occur in the paper from which I quote.

Dr. SANDERSON subsequently reaffirms the position here laid down. "In my preceding experiments," he says, "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 *Bacteria*"‡. And, again, with reference to another experiment:—"The result shows that ordinary air is entirely free

* From their deportment in boiling, I should infer that the air dissolved in an alkaline liquid is in a different physical condition from that dissolved in an acid liquid; and to this, in some measure, the difference of nutritive power may be due. I have been unable to find any experiments on the comparative absorption of air by acid and neutral liquids. The subject is, I think, well deserving of attention.

† Appendix to the Thirteenth Report to the Medical Officer of the Privy Council for 1871, p. 335.

‡ 'Appendix,' p. 338. Though Dr. SANDERSON speaks of "all nutritive liquids," if I understand him aright, he really tried but one, and that was a mineral solution, not an animal or vegetable infusion.

from living *Bacteria*"*. His general conclusion is that, as regards the development of *Bacteria* in organic liquids, "water is the contaminating agent."

Upon these experiments, and the conclusion drawn from them, an argument has been founded by Dr. BASTIAN†, which would be weighty were its basis sure. In reference to the Presidential Address of the British Association in Liverpool‡, he argues thus:—"Speaking of living *Bacteria* germs, Professor HUXLEY summed up by saying, 'considering their lightness, and the wide diffusion of the organisms which produce them, it is impossible to conceive that they should not be suspended in the atmosphere in myriads.' Had Profesor HUXLEY himself made some careful and discriminating experiments on this part of the subject, he might have found that the supposed impossibility of conception was entirely delusive. . . . What has been the subsequent progress of events? In the first place, it has been shown by Professor BURDON SANDERSON, myself, and others, that the living *Bacteria* germs are not diffused through the air to any appreciable extent; and this is now a very widely accepted doctrine, in spite of its being, as Professor HUXLEY imagined, an impossible conception." The "others" referred to by Dr. BASTIAN embrace among them, it is to be admitted, the celebrated naturalist COHN.

Dr. BASTIAN was quite correct in saying that the "doctrine" he enunciated was, at that time, "widely accepted." It is, nevertheless, one of those cases in which the general acceptance of a doctrine fails to establish its truth. It is, indeed, an entirely erroneous doctrine, founded, I will not say upon incorrect experiments, but on the misinterpretation of incomplete ones. I have already referred to this error, and should not do so now had it not been lately revived. The deportment of almost any sterilized animal or vegetable infusion exposed to common air will disprove the doctrine. It has been disproved repeatedly by experiments with melon-, turnip-, cucumber-, and hay-infusions, alluded to in this memoir. Such infusions, after having been sterilized by exposure for six or eight hours to the boiling-temperature, remain, if protected from the *Bacteria*-germs of the air, for ever barren; but when infected spontaneously, or purposely, by atmospheric germs, they are found, within eight and forty hours after such infection, thickly crowded with *Bacteria*. That London air is laden with living *Bacteria* germs is as certain as that London chimneys are laden with smoke. What Dr. SANDERSON's important experiments really prove is, that a mineral solution competent to nourish the *Bacteria* after they have been fully developed is not competent (or, rather, but very feebly competent) to effect the transfer from the germ state to that of the finished organism. It can feed the chick, but it cannot hatch the egg. As I have already expressed it, the experiment proves, not the absence of *Bacteria*-germs from the air, but the inability of the mineral solution to develop them.

Another experiment, described by Dr. SANDERSON in the paper above referred to, is this:—"A glass rod was charged with *Bacteria* by dipping it into a solution on the surface of which there was a viscous scum, consisting entirely of these bodies imbedded in a gelatinous matrix. The rod was allowed to dry in the air for a few days. It was

* 'Appendix,' p. 339. † 'Evolution,' p. 44. ‡ Brit. Assoc. Report, 1870.

then introduced into boiled test-solution contained in a superheated glass. On February 6th the liquid was already milky, and teemed with *Bacteria*.

"To determine the effect of more complete desiccation, an éprouvette containing one cubic centimeter of cold water, previously ascertained to be zymotic, was evaporated to dryness in the incubator, and kept for some days at a temperature of 40° Cent. On February 20th 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 2nd, when it contained numerous *Torula*-cells, but no trace of *Bacteria*. It therefore appears that the germinal particles of *Bacteria* are rendered inactive by drying without the application of heat.

"As it appeared probable," continues Dr. SANDERSON, "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 the éprouvette, and dried for some days in the incubator. The éprouvette was then charged with both boiled and cooled PASTEUR'S solution, and plugged with cotton-wool. On March 11th the liquid was slightly hazy, but was found to contain no trace of *Bacteria*. The haziness was due to the presence of *Torula*-cells in great numbers. It thus appeared that fully-formed *Bacteria* are deprived of their power of further development by thorough desiccation."

To the present hour these experiments are quoted as conclusive in reference to the influence of desiccation. They are quoted, moreover, as applicable not only to the developed *Bacterium*, but also, without restriction, to the germs from which *Bacteria* spring. "To maintain," says Dr. BASTIAN *, "his Panspermism in the face of his own experiments, SPALLANZANI was compelled to assume that the germs of the lower infusoria do possess this seed-like property of developing after desiccation. Modern science, however, declares that they have no such property. We are told most unreservedly by Professor BURDON SANDERSON, not only that the germinal particles of *Bacteria* are rendered inactive by thorough drying, without the application of heat, *i. e.* by mere exposure to air for two or three days at a temperature of 104° Fahr., but also that fully formed *Bacteria* are deprived of their power of further development by thorough desiccation." In this unqualified sense the conclusion is certainly an erroneous one; and that it is so has been proved by experiments far more stringent than those of Dr. SANDERSON. I could cite a multitude of such experiments, but a reference to one or two of them will here suffice.

A small bunch of old Heathfield hay was washed with distilled water, which was received into a champagne-glass. The glass was placed on a stove until the water had all evaporated, and the dried residue was permitted to remain upon the stove for several days. Dr. SANDERSON'S drying temperature was 104° Fahr., mine was 120° Fahr., and my period of drying was longer than his. Scraping a little of the dry sediment treated as above from the bottom of the champagne-glass, I infected with it a bulb containing

* 'Evolution,' p. 156.

hay-infusion which had been completely sterilized by eight hours' boiling. When infected, the infusion was brightly transparent, but forty-eight hours after its infection it was teeming with *Bacteria*. With regard to the doctrine that these organisms arise from "dead organic particles," instead of from living germs *, no scientific man at the present day ought, I submit, to be called upon to spend a moment's thought upon it.

One other reference will suffice. I have had bundles of hay hung up for seven or eight weeks in the hot rooms of the Turkish Bath in Jermyn Street, and exposed during the whole of this time to a temperature of 140° and upwards. The germs adherent to this hay were not killed by even this amount of desiccation. When a sterilized animal or vegetable infusion was infected with them they gave birth in the usual time to swarms of *Bacteria*.

§ 22. *Sterilization by discontinuous Heating.*

Keeping the distinction between germs and developed organisms here insisted on, and the probable changes that occur in passing from the one to the other, clearly in view, I have been able to sterilize with infallible certainty the most obstinate infusions referred to in this paper; and this has been accomplished without either raising the temperature of the infusions beyond their ordinary boiling-point, or inordinately prolonging the application of heat. The infusions may be sterilized by a temperature even below that of boiling water, while the time of its application may be but a minute fraction of that resorted to in some of the foregoing experiments.

It is an undisputed fact that active *Bacteria* are killed by a temperature far below that of boiling water. It is also a fact that a certain period, which I have called the period of latency, is necessary to enable the hardy and resistant germ to pass into that organic condition in which it is so sensitive to heat. There can hardly be a doubt that the nearer the germ approaches the moment when it is to emerge as the finished organism, the more susceptible it is to that influence by which that organism is so readily destroyed. We may learn from experience, aided by the power of search which the concentrated luminous beam places in our hands, what is the approximate time required for the Bacterial germ to pass into the *Bacterium*. Say that it is twenty-four hours. Supposing the heat of boiling water, or even a lower heat than that of boiling water, to be applied to the germ immediately before its final development, when all its parts are plastic, when it is, in short, on the point of reaching a stage at which a temperature of 140° Fahr. is demonstrably fatal. It is in the highest degree probable that a temperature of 212°, or of 200°, or, indeed, a temperature of 150° if applied sufficiently often or for a sufficient length of time, will prove fatal to the germ, and prevent the appearance of the still more sensitive organism to which the germ is on the point of giving birth.

Here, at all events, we have a theoretic finger-post pointing out a course which experiment may profitably pursue. It is not to be expected that the germs with which our

* 'Evolution,' p. 44.

infusions are charged all reach their final development at the same moment. Some are drier and harder than others, and some, therefore, will be rendered plastic and sensitive to heat before others. Hence the following procedure.

Four and twenty small retorts were charged with hay-infusions on the 1st of February, and subjected morning and evening to the boiling temperature for one minute. The last heating took place on the evening of the 3rd of February. The retort-necks had been plugged with cotton-wool; the air within them, however, had not been filtered, and there was comparatively little care bestowed on their preparation. After the final heating they were abandoned to the temperature of a room kept close to 90° Fahr. A series of similar retorts charged at the same time with the same infusions were boiled continuously for ten minutes, plugged while boiling, and placed in the same warm room. Two days after their preparation the retorts last mentioned had, without a single exception, given way to turbidity and scum. On the other hand, twelve of the twenty-four retorts which had been subjected for a much shorter period to the discontinuous boiling remained permanently brilliant and free from scum.

On the 1st of February eight pipette-bulbs were charged with two hay-infusions, four bulbs being devoted to each. The air above the infusions was the unfiltered air of the laboratory. They were subjected to the temperature of boiling water for a minute; at the same time four other bulbs containing the same infusions were boiled continuously for ten minutes and suspended beside their neighbours. Twelve hours subsequent to their first brief heating the eight bulbs were perfectly brilliant, and while in this condition they were again subjected to the boiling temperature for a minute. On the evening of the same day they were subjected to the boiling temperature for half a minute, and on the following morning the process was repeated. Two additional heatings of the same brief character were resorted to. The result of the whole experiment was that two days after their preparation the four bulbs which had been boiled for ten minutes were found turbid and covered with scum, while two months after their preparation the eight bulbs whose periods of boiling added together amounted only to four minutes were perfectly brilliant and free from scum.

The reason of this procedure is plain. By the first brief application of heat the germs, which are at that moment plastic, are killed; and before any of the remaining germs can develop themselves into *Bacteria* they are subjected to another brief period of heating. This again kills such germs as are sufficiently near their final development. At each subsequent period of heating the number of living germs is diminished, until finally they are completely destroyed. The infusion, if protected from external contamination, remains for ever afterwards unchanged, although, when living *Bacteria*, a sprig of hay, or even the dry dust particles of the laboratory are sown in it, the sterilized liquid shows its power both of enabling the fully developed organism to increase and multiply, and of developing the desiccated Bacterial germ into multitudinous Bacterial life.

On the same date an experiment was made with a series of pipette-bulbs, whose necks were so bent and plugged with cotton-wool that no impurity from the wool could fall into the infusion. Four bulbs were charged with an infusion of Heathfield and four with an infusion of London hay, samples of the same infusions being introduced at the same time into another series of bulbs which were plugged like their neighbours and subjected continuously to the boiling temperature for ten minutes. The eight bulbs first referred to were, on the contrary, discontinuously boiled, the sum of their periods of boiling being four minutes. The result is that while the entire series of bulbs boiled for ten minutes gave way within forty-eight hours after their preparation, seven out of the eight bulbs which had been subjected to discontinuous boiling remained permanently brilliant.

On the 3rd of February, with the view of testing the new method still further, infusions of our most refractory kinds of hay were prepared. There were five bulbs of neutralized Guildford infusion, and five of a neutralized infusion formed from a mixture of old Colchester and old Heathfield hay. Two bulbs of each infusion were at the same time charged and subjected to the boiling temperature for ten minutes. The ten bulbs first mentioned were never raised to the boiling temperature at all, the maximum to which they were exposed being some degrees below their boiling-point. The result is that while every one of the four bulbs boiled for ten minutes has become turbid and covered with scum, one only of the ten discontinuously heated bulbs has given way; nine of them remain as brilliant as at first.

It is obvious from what has gone before that two hundred and forty minutes might have been substituted for ten minutes without altering this result. Five minutes of discontinuous heating can accomplish as much as five hours continuous heating.

On the same date three bulbs charged with an acid infusion of London hay were subjected to the same discontinuous treatment. They all remain brilliant to the present hour.

On the 7th of February four of COHN's tubes were charged with turnip-infusion, which was heated discontinuously night and morning up to a temperature of 205° Fahr. The total time during which they were exposed to this temperature was about three minutes. They were permanently sterilized, and exhibit a singular brilliancy to the present hour.

The discontinuous method of heating has also been applied with success to the closed chambers. One mode of operation is this:—An oil-bath is heated to a temperature of 300° Fahr. The charged test-tubes of the closed chamber are then plunged in the oil, which clasps the tubes to the level of the surface of the infusion. They are either raised to incipient boiling and then removed, or suffered actually to boil for thirty seconds and then removed. Another mode of heating is this: instead of being plunged into hot oil, the test-tubes are plunged for two or three minutes into boiling water, taken out, wiped dry, the actual boiling being finished by a spirit-lamp. This is a very handy method, and more under the experimenter's control than the oil-bath. When the latter is employed the infusions sometimes in great part waste themselves by leaping from their tubes; but the spirit-lamp enables us to humour the infusions by occasionally withdrawing the flame and moderating the ebullition. The lamp, of course, may be employed alone without the preliminary immersion of the tubes in hot water. Usually the process of heating is repeated at intervals of twelve hours, but in the case of very nutritive infusions in a very warm room the interval ought to be shorter. Practice must inform the experimenter on this point. The reheating must always occur before the infusions show the slightest tendency to change.

In the early days of February a closed chamber of six tubes was treated in the manner here described. Three of the tubes were charged with strong turnip and the three others with strong artichoke-infusion. After two days discontinuous heating night and morning, they were allowed to remain undisturbed in the warm room. The six tubes remain perfectly brilliant to the present hour.

On the 12th of February a closed chamber of three tubes was charged with cucumber-infusion. Heated discontinuously in the manner described, and abandoned afterwards to a warm temperature, the three tubes remained perfectly sterile.

Any process competent to sterilize very old hay can sterilize with greater ease any other infusion. The fact, therefore, that only a few days ago three closed chambers charged with our most refractory hay-infusions were sterilized by discontinuous heating proves the power of the method over infusions of all kinds.

By this method very instructive comparative experiments might be made, and the resistant power of different germs might be expressed in terms of the heatings necessary for their sterilization. I possess, for example, two test-tubes, containing the same infusion and associated with the same closed chamber, one of which has been heated five times and the other six. The former is quite turbid, while the latter is perfectly clear. In this case five heatings had left some of the more resistant germs still unkilled, which were destroyed by the sixth heating. Of two other tubes charged with a different infusion, one has been heated seven times and is now full of life; the other has been heated eight times and is perfectly barren.

With due care the method of sterilization here described is infallible, however highly infective the surrounding atmosphere may be. But here, as elsewhere in these difficult inquiries, the sagacity which comes in great part from nature, the skill which comes from training, and the care which ought to root itself in his moral constitution are all necessary to save the experimenter from error and to lead him to the truth.

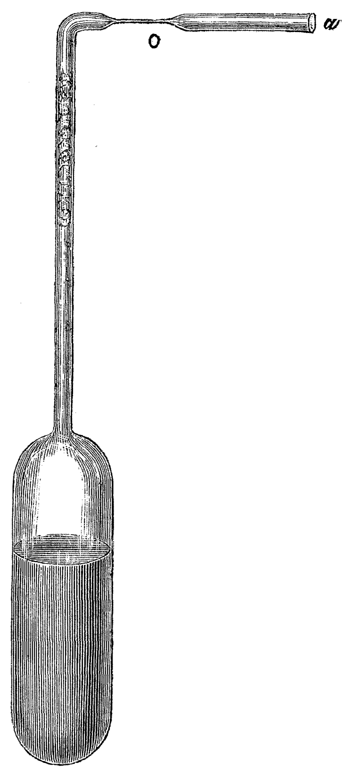
§ 23. *Mortality of Germs through defect of Oxygen produced by Exhaustion with the* SPRENGEL *Pump.*

An equally striking mode of sterilization is now to be described. The crowding together of the organisms so as to form in a multitude of cases a heavy, corrugated, fatty scum upon the surface of the infusions obviously indicated that air was a necessity of their life. In some cases the oxygen dissolved in the infusions sufficed to enable the *Bacteria* to cloud them from top to bottom; but in many cases they gathered at the top, and formed there a living layer through which no oxygen could pass to the liquid underneath, which, thus surmounted, remained as clear as water. The observation of these facts, and many others of a similar bearing, suggested inquiry into the effect which the more or less perfect withdrawal of the air from the infusions would have upon the development of life.

A few experiments with an ordinary air-pump were, in the first instance, made. The necks of a series of bulbs charged with turnip-infusion were drawn out at the middle to a tube of very small diameter. The open end of the neck being connected with the air-pump, the bulbs were exhausted. In some cases, to render the removal of the air more perfect, hydrogen was admitted into the bulb and was afterwards withdrawn by the air-pump. Before they were detached from the pump the bulbs were immersed in luke-warm water. They boiled freely, and after a minute's ebullition the narrowed necks were hermetically sealed. The bulbs were then submerged in cold water, which was gradually raised to 212° F. and kept boiling for ten minutes; they were afterwards removed and placed in a room with a temperature of about 90° Fahr.

Four bulbs were thus treated in a preliminary experiment on the 7th of March. Two of them remain crystal clear to the present hour; the two others became cloudy, but remained entirely free from scum. The cloudiness, I may add, was barely perceptible, but it was perfectly distinct to the practised eye.

Fig. 11.



By such means, however, the removal of the air must have been more or less imperfect, and I therefore resorted to the far more effective Sprengel pump. To connect them with the pump, the bulbs were thrown into the form represented in fig. 11. After the neck of the bulb had been plugged with cotton-wool it was bent at right angles above the plug, and a portion of it was drawn out to a tube of capillary diameter, represented at *o*. The end *a* was connected with the Sprengel pump, and after the exhaustion had been continued for the required interval, the neck of the bulb was sealed at *o*.

On the 14th of March three bulbs charged with the turnip-infusion, from which the air had been as far as possible removed by the ordinary air-pump, were connected with the Sprengel, which continued the exhaustion uninterruptedly for three hours. The air dissolved in the liquid freely escaped from it at first, and it continued to appear in minute bubbles long after the exhaustion had reached a considerable degree of perfection. The drawn-out necks of the bulbs being hermetically sealed, the infusion within them was maintained as before for ten minutes at the boiling temperature.

It will be remembered that when the infusion and the air above it possessed their ordinary supply of oxygen, 180 minutes' boiling failed to sterilize the turnip-infusion. Here, when the air was withdrawn from the liquid, exposure for one eighteenth of the foregoing interval sufficed to produce perfect barrenness. The infusion in the three bulbs here operated on remains to the present hour clear in body and perfectly free from scum.

On the 15th of March seven bulbs charged with infusion of turnip were treated in the manner just described, being purged of their air by three hours' action of the Sprengel pump, and boiled for ten minutes afterwards. Six out of the seven remain perfectly pellucid.

On the 16th of March the result was still further verified. Seven bulbs were then charged with turnip-infusion, exhausted first by the air-pump, and afterwards by five hours' action of the Sprengel pump. Hermetically sealed and boiled as before, six out of the seven remain as clear as distilled water.

On the 20th of March seven bulbs were charged with infusion of cucumber, and subjected to the action of the Sprengel pump for seven hours. They were afterwards treated in the manner just described. They were all completely sterilized.

On the 27th of March three bulbs were charged with cucumber-infusion and subjected to the action of the Sprengel pump for five hours. One of them was subsequently boiled for five minutes, another for one minute, while the third was left unboiled. This third tube became faintly cloudy, but the two others remain perfectly free from life.

This result invited repetition. On the 29th accordingly six bulbs of cucumber-infusion were exhausted for five hours, and afterwards sealed and boiled for a single minute. Five of the six bulbs remained permanently clear; one became cloudy.

On the 30th of March six bulbs containing turnip-infusion were exhausted for five hours and boiled afterwards for a minute. Five remain perfectly clear, one has become muddy.

On the 6th of April five bulbs of beef-infusion were subjected for three hours to the action of the Sprengel pump and boiled for a minute afterwards. They all remain brilliant.

On the 7th of April five bulbs of mutton-infusion were treated like the beef-bulbs, being exhausted for three hours and boiled for a minute. All remain clear. This experiment was repeated and confirmed on the 20th of April.

On the 14th of April three bulbs of pork-infusion were exhausted for four hours and boiled for a minute. They all remain pellucid.

On the 17th of April four bulbs of accurately neutralized urine were exhausted for five hours and boiled for a minute. Three of them remain bright; one has become cloudy.

This does not exhaust the list of instances. Many other infusions have been sterilized by this method since the 17th of April.

It is perfectly certain that in most, if not all, of these cases 200 minutes' boiling would have proved insufficient to sterilize the infusion if it had been supplied with air.

Here the question naturally arises:—What would happen if the bulbs were exhausted and left unboiled? Probably with sufficiently perfect exhaustion all infusions would be sterilized. But in the trials I have thus far made some of the unboiled infusions have become cloudy, while others have remained clear. Thus three bulbs of mutton-infusion exhausted for four hours, two bulbs of beef-infusion exhausted for three hours, four bulbs of pork-infusion exhausted for four hours and left unboiled remain as transparent and as free from life as their boiled companions. Various other instances of sterilization without boiling might be cited. On the other hand, three bulbs of neutralized urine, exhausted for five hours and left unboiled, became cloudy. A case of cucumber-infusion which behaved similarly has been cited above. It is difficult, if not impossible, to remove from the infusion and the space above it the last traces of air; and when backed by a highly neutritive liquid an infinitesimal residue of oxygen can develop a sensible amount of life. I may add that I have tested the exhaustion of some of the cloudy bulbs, and have found it in every case defective.

The foregoing instances sufficiently illustrate the dependence of the organisms here under review upon the supply of oxygen*. I think it probable that the principle thus indicated is capable of useful and extensive practical application.

§ 24. *Mortality of Germs through defect of Oxygen consequent on boiling the Infusion.*

Long prior to these experiments with the Sprengel pump, the influence of atmospheric oxygen on the life of these organisms had been brought home to me. It revealed itself in a striking manner in experiments with infusions purged of air by boiling, the vessels containing them being carefully sealed during ebullition. At a time when the atmosphere of our laboratory was so laden with infection that no escape for animal or vegetable liquids introduced in the usual way into closed chambers was possible, it was perfectly easy to keep the same infusions pellucid for an indefinite time in vessels purged of air by boiling and properly sealed. I will give a few out of the multitude of examples that might be cited in proof of this statement.

On the 2nd of October fourteen of our ordinary retort-flasks (fig. 4) were charged with a neutralized infusion of hay. They were boiled for three minutes in an oil-bath, and hermetically sealed whilst boiling. Thirteen out of the fourteen tubes remained perfectly barren, retaining for months their pristine colour and transparency.

On the 18th of November six retort-flasks were filled with turnip-, five with cucumber-, five with beetroot-, and four with parsnep-infusion. The six turnip-flasks remained permanently pellucid, yielding a clear water-hammer ring. The five beetroot-flasks remained also permanently barren, all yielding the water-hammer sound. Of the parsnep-flasks, two became turbid, but two remained clear. Of the cucumber-flasks, three became cloudy, while three remained permanently clear. Neither in the case of the parsnep

* In search of this gas they sometimes rise into the liquid film which covers the interior of the bulb to the height of an inch and more above the surface of the liquid, forming within the bulb a gauzy scum which appears as if lifted by capillary attraction. Their mode of nutrition must therefore be very different from that of *Torula*-cells, as revealed by the excellent researches of PASTEUR.

nor in that of the cucumber could the water-hammer sound be obtained from the cloudy flasks, and when their sealed ends were broken under water, the vacuum was found defective. In the clear tubes, on the contrary, it was found practically perfect.

Again, on the 20th of November, seventeen retort-flasks were charged with infusions of turnip, cucumber, and parsnep. They were boiled for three minutes in an oil-bath, and carefully sealed while boiling. The six turnip-flasks remained permanently clear, maintaining for months their sharp water-hammer sound. Of the five parsnep-flasks, one became turbid and four remained permanently clear. These latter only yielded the water-hammer sound. The turbid flask, on the contrary, when shaken yielded no such sound, and when its sealed end was broken under water, its vacuum proved defective. Of the six cucumber-flasks, two became turbid, the remaining four being perfectly clear. On breaking their sealed ends under water, one third of one of the turbid flasks and one fourth of the other remained unfilled by the liquid.

On the 6th of December eighteen retort-flasks were charged with cucumber-infusion. They were boiled for the usual time, that is three minutes, extreme care being taken to seal them during the issue of the steam. The water-hammer sound in all these flasks was particularly sharp and clear. Exposed to a temperature of 90° Fahr. for many weeks, seventeen of them remained perfectly pellucid; while the same infusion in a sealed bulb with filtered air above it and dissolved in it swarmed with life after boiling for sixty times the interval here found effectual.

On the day subsequent to its preparation, one of these well-exhausted flasks was unexpectedly found turbid and covered with scum. But on examining the sealed end it was found snipped off. The laboratory air had thus entered the flask and given birth to the observed organisms. Failures of this sort have a demonstrative force greater even than successes; they render so obviously plain the external source of the contamination.

It is worth saying here that the observation just recorded was of frequent occurrence. The fineness of the sealed points of our retort-flasks renders them very liable to be snipped off if they are not handled with care. After preparation they are usually suspended on a wire or on a wooden support; and in frequent instances, after such suspension, I have found a flask differentiating itself by thick turbidity from a number of perfectly pellucid neighbours, the yielding of the flask being immediately traced to the fracture of its sealed end.

With the view of showing how readily, unless extreme care is taken, contamination may enter hermetically sealed vessels, the following experiment was made on the 6th of December. Four retort-flasks were charged with the cucumber-infusion, boiled for the usual time and sealed, not during the outrush of the steam, but a moment after ebullition had ceased. On the 9th of December three of these four flasks were faintly but distinctly turbid. The reason is obvious. On the cessation of the ebullition, a momentary condensation of the steam above the infusion caused an indraught—slight, no doubt, but still sufficient to contaminate or vivify the infusion.

The source of the contagium was also indicated by the following experiments. A large number of retort-flasks, embracing infusions of snipe, wild duck, partridge, hare, rabbit, mutton, turbot, salmon, whiting, mullet, turnip, and hay, had remained over from my stock of 1875. After a year's exposure to the temperature of our warm room not one of these flasks showed the slightest trace of turbidity or life. On the 7th of December the sealed ends of forty of them were snipped off in the laboratory. Five days afterwards twenty-seven of them were found swarming with organisms—a considerably higher percentage than that obtained by the same process in the same laboratory a year previously.

It is needless to dwell with any emphasis on the obvious inference from all this, namely, that the contagium is external to the infusions (that it is, something in the air), and that at different times we have different amounts of aerial interspace free from the floating contagium.

§ 25. *Critical Review of the last two Sections.*

It has been my desire and aim throughout this inquiry to free it as much as possible from uncertainty and doubt. I have tried to render the facts safe by laborious repetition, and to render the interpretations of those facts secure by close and constant criticism. Thus, in reference to our present subject, I had to put to myself very definitely the question whether the permanent clearness of an infusion exposed to a very moderate amount of heat, after having been freed from air by boiling or by the Sprengel pump, was really due to the destruction of the germs in the infusion. Even in a highly infective atmosphere from three to five minutes' boiling in an oil-bath suffices to sterilize our retort-flasks, while it is perfectly certain that exposure to the boiling temperature for fifty times this interval fails to kill the germs of an infusion containing a good supply of atmospheric air. A similar remark applies to our experiments with the Sprengel pump. I asked myself whether in these cases the life of the germs was not suspended merely, instead of being destroyed. It was quite conceivable that germs endowed with vital power, ready to act under proper conditions, might still exist in our hermetically sealed flasks, although the entire absence of oxygen rendered their further development impossible.

That something more than a mere temporary hindrance to development is here involved was, however, proved by many of the experiments just recorded. These experiments showed that after hermetically sealed flasks had remained pellucid, not only for days but for weeks and months, and in some cases for more than a year, when their sealed ends were broken off, even in ordinary air, they by no means invariably showed signs of life afterwards. Many of them remained permanently barren while copiously supplied with oxygen.

Special experiments were, however, made to illustrate this point. First of all, as I have already recounted, hermetically sealed retort-flasks were opened in one of our lower store-rooms, and though supplied with oxygen from this source they showed subsequently no signs of life. A considerable number of retort-flasks had also their sealed ends broken off in the midst of a spirit-lamp flame. It is known that gunpowder can be dropped through such a flame without ignition; and in a few rare instances the infusions which had their first supply of air thus passed through a flame showed subsequent signs of life. In such cases the germinal matter had been sucked so rapidly through the flame that it escaped destruction; but in the vast majority of instances the sterilized infusions remained sterile.

Mechanical arrangements were also made for the breaking off of the sealed ends in a receiver filled with filtered air. But it is by no means easy to perfectly cleanse from infectious matter the instruments used in such experiments, though with sufficient practice this might certainly be done. The consequence was that some of the flasks opened in filtered air yielded subsequently. The following is an illustrative case:—On the 3rd of January ten flasks were charged with cucumber-, turnip-, artichoke-, and melon-infusions. They were boiled for the usual time, sealed during ebullition, and exposed afterwards to a warm temperature. Their sealed ends were then broken off by a mechanical contrivance placed in a receiver containing filtered air. The two artichoke-flasks remained permanently barren afterwards. Of the two melon-flasks, one remained barren and the other developed life. Of the two cucumber-flasks, one became turbid, the other remained clear. Of the four turnip-flasks, two became turbid and two remained clear. Out of the ten flasks, therefore, all freely connected with the external air, six remained permanently barren. By further practice barrenness in almost every instance has been secured. The conclusion, I think, is obvious. It is not the heat alone that destroys these germs, for fifty times the amount of heat will not accomplish this when oxygen in due quantity is present: the heat must be aided by the withdrawal of the oxygen.

§ 26. *Mortality of Germs through excess of Oxygen.*

The foregoing remarks lead naturally to a brief reference to the important experiments of M. Paul Bert* on the toxic influence of compressed oxygen. From the imperfect account of these experiments which first reached me, I inferred that the germs of putrefaction had been destroyed by mere mechanical pressure, and more than a year and a half ago I placed turnip-infusions in strong iron bottles, and subjected them for several days to an air-pressure of twenty-three atmospheres. When taken from the bottles the infusions were found one and all swarming with life. Last October I made a series of similar experiments with infusions of hay and turnip, subjecting them for several days to an air-pressure of twenty-seven atmospheres. When taken from their iron bottles the infusions were found one and all teeming with *Bacteria*.

I then resorted to pure oxygen, and found the same to be true of my infusions that M. Paul Bert had found true of his flesh, moist bread, boiled starch, strawberries, cherries, wine, and urine. Pressures varying from twenty-seven atmospheres to ten

* Comptes Rendus, vol. lxxx. p. 1579.

atmospheres of oxygen were employed. In all cases, however long the pressure was continued, or however favourable to putrefaction the surrounding temperature might be, the infusions (which embraced those of beef, mutton, and turnip) were found, when taken from the bottles, as clear as crystal and entirely free from life. It required, indeed, long subsequent exposure to the common air to infect infusions which had been thus surcharged with oxygen. Other bottles containing the same infusions were simultaneously subjected to a like pressure, not of oxygen, however, but of atmospheric air. When removed from the bottles they were one and all found in a state of putrefaction and swarming with life.

Thus when oxygen is wholly withdrawn from organic infusions, the life with which we are here concerned ceases. When, on the other hand, the gas is in considerable excess, it becomes a deadly poison to organisms which, in moderate quantities, it sustains. As in the case of temperature, so in regard to the supply of oxygen, there is a median zone favourable to the play of vitality, beyond which, on both sides, life cannot exist.

The present memoir virtually ends here; but I will append a few brief sections which, though incomplete, are not without instruction.

§ 27. *Experiments on neutralized Urine.*

I have already communicated to the Royal Society the result of some experiments made with this liquid*, in which the potash employed for neutralization was subjected to a temperature of 220° Fahr. The alkali was contained in tubes drawn out finely at the end, which were introduced into the flasks containing the urine, and broken by shaking after the acid urine had been completely sterilized by heat. The cases were exceedingly rare in which life showed itself in the urine thus neutralized. The preponderance of sterilized flasks over unsterilized ones was enormous.

In the experiments now to be glanced at, neither the urine nor the potash was raised to a temperature higher than 212°. Wishing to ascertain how the refractory germs of our laboratory would fare in neutralized urine, on the 16th of February I had five pipette-bulbs charged with the liquid. It was neutralized by caustic potash, which on boiling produced copious precipitation. It was afterwards filtered and rendered very transparent. The bulbs had been well cleansed, filled with one third of an atmosphere of filtered air, hermetically sealed, and exposed afterwards to the heat of a Bunsen flame. They were charged with the urine by breaking off their finely drawn out points in the body of the liquid. They were then again sealed, and subjected to the boiling temperature for ten minutes.

Not one of these bulbs remained sterile. Two days subsequent to their preparation they were all swarming with organisms.

Three other bulbs were on the same occasion charged with precisely the same infusion, only instead of being associated with air they were well purged of air by five minutes' boiling in an oil-bath. While the steam was issuing they were hermetically sealed.

* Proceedings, vol. xxv. p. 457.

Not one of these bulbs has proved fruitful. They are all as brilliant and as free from life as they were after they had passed through the filter.

The difference here indicated is worthy of notice. In the one case five minutes' action completely sterilizes; in the other ten minutes' action fails to do so. This latter interval indeed might be multiplied twentyfold and still prove ineffectual. In the one case the process of boiling purged the liquid of its air; in the other case the air was retained within and above the liquid. The case therefore connects itself with our former illustrations.

On the 21st of February six bulbs were charged with fresh urine carefully neutralized and boiled for five minutes in an oil-bath. Of the six flasks, four remain perfectly clear and brilliant, one is slightly cloudy, and one turbid.

The urine here referred to was neutralized in our own laboratory; but as the importance of accurate neutralization has been much insisted on, I wished to check myself. At my request, therefore, Dr. DEBUS was good enough to send me from Greenwich a quantity of urine carefully neutralized by him. On the 1st of March seven retort-flasks were charged with the neutralized liquid. These were boiled for five minutes in an oil-bath and sealed during ebullition. Three of these flasks have become turbid, but four remain perfectly clear.

On the 5th of March Dr. WILLIAMSON was good enough to send me a supply of neutralized urine collected in a public urinal in University College. The colour was very deep, the odour was very bad, and the precipitation on boiling very copious. Fourteen retort-flasks were charged with this liquid on the 6th of March. Seven of them have gone bad, but seven of them remain clear.

On the 10th of March Dr. FRANKLAND was good enough to send me a supply of urine neutralized by himself. It was introduced into four retort-flasks, which, like the others, were boiled for five minutes in hot oil and sealed during ebullition. None of these flasks have shown the slightest sign of yielding. The liquid within all of them is as brilliant as it was when first introduced.

In every case here mentioned the liquid, after boiling, was exposed for several days to a temperature of 50° C.

The conflict described in the foregoing pages and the search for principles to reconcile the results occupied me too long to permit of my doing more than break ground on the subject of urine. I entertain, however, a strong opinion that by a little practice with this liquid, its sterilization by five minutes' boiling might be rendered certain in every case. As the experiments stand, they sufficiently negative the conclusions of Dr. BASTIAN, who has brought forward the deportment of neutralized urine as a specially convincing illustration of spontaneous generation. Nor are they in accordance with the statement of M. PASTEUR, that in neutralized urine, subjected only to the ordinary boiling temperature, organisms in the majority of cases ("le plus souvent") appear.

§ 28. *Hermetically sealed flasks exposed to the sun of the Alps.*

A remark of Dr. BASTIAN'S, wherein he refers to the power of the actinic rays of the sun to promote spontaneous generation*, caused me to take with me last summer to Switzerland a number of flasks hermetically sealed with special care and charged with infusions of various kinds. Eighty of them were carefully packed in sawdust in London; but on my arrival at the Bel Alp, which stands at an elevation of some 7000 feet above the sea, I found only forty-five of them unbroken. They were thus distributed:—

Beef	12 flasks.
Mackerel	12 „
Turnip	12 „
Fowl	9 „

For ten days of the splendid summer with which we were favoured during a portion of last July, these flasks were exposed daily to the sunlight upon the roof of the Bel Alp hotel. The sky during many of these days was of a deep and cloudless blue; and certainly in London the actinic rays never approached the power of those here brought to bear upon the infusions. The temperature for many hours of each day was about 120° Fahr. Every evening, when the thermometer had fallen to about 70°, the flasks were removed and suspended above the kitchen-range of the hotel, the temperature generally varying throughout the night from 70° to 80° Fahr. Such variations of temperature, it may be remarked, are deemed by Dr. BASTIAN favourable to spontaneous generation.

After the sunny weather had disappeared, the flasks were allowed to remain for three weeks suspended in the kitchen, with occasional exposures to the sun; the average temperature of the kitchen where the flasks were hung was about 90° F. The result of the observations was that not one of these forty-five flasks yielded the slightest evidence of spontaneous generation. From first to last they all continued as limpid as distilled water.

The sealed ends of these flasks were afterwards snipped off under various circumstances, some on the Sparrenhorn, some on the glacier, some in the Massa Gorge, some amid the hair of my own head, and some in the rooms of the hotel. Many of them, moreover, were infected with water of various kinds—spring-water, lake-water, and glacier-water. It is not my object to give a detailed account of these experiments, but simply to say that it was not lack of nutritive power on the part of the infusions which prevented the appearance of organisms in the first instance; for when brought into contact with infectious matter every one of the flasks showed its power of sustaining and multiplying life.

§ 29. *Remarks on Hermetic sealing.*

A few brief remarks on this subject may, I think, be fitly interpolated here. Hermetic sealing during ebullition is an operation requiring some apprenticeship to perform it aright. The neck of the flask ought to be so narrow that the pressure of the steam within shall be always sensibly greater than that of the atmosphere without. This

* Nature, vol. iii. p. 247.

condition would be readily fulfilled if the liberation of the steam were absolutely uniform, and not by fits and starts. But it never is uniform, and if the channel through which the steam issues be wide, it is scarcely possible to avoid regurgitation. Sometimes the pressure within is above that of the atmosphere, and steam freely issues; but at the next moment, through liquid adhesion to the flask and partial condensation above, the internal pressure may be below that of the atmosphere and permit air to enter. This alternate triumph of the inner and the outer pressure may be rendered plainly evident by the motions of the water condensed in the neck of the flask. The liquid acts as an index which moves to and fro, sometimes forward, sometimes backward, as the pressure varies. It is quite evident that contamination may be, and it is quite certain that contamination has been, thus introduced into flasks reputed to be free from air.

Even with considerable care and highly disciplined manipulatory skill success is not invariable. Ten per cent. is not at all a large allowance to set down as defective in ordinary hermetically sealed flasks. The recent opening of about two hundred flasks employed in my earlier experiments under water and under caustic-potash solution furnishes the basis of this conclusion. Even in a comparatively pure atmosphere success does not in every instance attend the experimenter. At Kew, for example, on the 8th of January, thirteen retort-flasks were charged with infusions of cucumber, melon, beef, and sole. Twelve out of the thirteen remained perfectly limpid, but one of them (a cucumber-flask) became distinctly cloudy, and this one alone refused, when tested, to yield the water-hammer sound.

§ 30. *Experiments with Turnip-cheese infusions.*

I am unwilling to omit all reference to experiments which have cost considerable labour, and which, though they have not been repeated and controlled to the extent that I could wish, contribute nevertheless to our knowledge of the present question. This unwillingness causes me to introduce here, in the briefest manner possible, a reference to a series of experiments made with turnip-cheese infusions, so frequently cited by Dr. BASTIAN as offering a conspicuous proof of the doctrine of spontaneous generation.

The experiments to which I refer were made in part with closed chambers and in part with hermetically sealed retort-flasks. The specific gravity of the infusions varied from 1008 to 1012. The cheeses employed were Cheshire, Cheddar, Gloucester, Dutch cheese, American cheese, Roquefort, and Parmesan, the quantity varying from half a grain to two grains for every ounce of the infusion. The cheese being first well triturated in a mortar, so as to render its particles very minute, was intimately mixed with the infusion, which was then boiled for a few minutes and passed through a filter. The filtered liquid was then introduced into its closed chamber, and boiled there for five minutes.

Sixteen such chambers were employed, one of them containing twelve test-tubes, each of the others only three. There were therefore fifty-seven test-tubes in all. The result of the experiment was, that out of the fifty-seven tubes twenty-seven became turbid in a few days, while thirty remained for months without sensible alteration*.

A considerable number of retort-flasks were charged at the same time with the same infusions, and boiled for five minutes in an oil-bath. The great majority of these flasks remained perfectly intact.

Here, then, as elsewhere, the ground on which the doctrine of spontaneous generation has sought to plant itself slips from under it; for assuredly the scientific mind will attribute to other causes than to it the production of organisms in the minority of cases above referred to.

One likely cause may here be signalized and illustrated. The experiments of SPALLANZANI on the action of heat upon seeds are well known, and they have been frequently cited by Dr. BASTIAN in support of his favourite thesis that the briefest exposure to the temperature of 212° Fahr. suffices to destroy all living matter. I have repeated many of SPALLANZANI'S experiments, and will here briefly refer to one series which bear upon the present point. Peas, kidney-beans, cress- and mustard-seed were tied up in small calico bags, and boiled for intervals varying from half a minute to five minutes. They were then carefully sown in flower-pots filled with well-prepared earth, and placed in a shed kept at a temperature of 70° Fahr. An unboiled sample of every seed was sown at the same time beside the boiled ones. The unboiled seeds sprouted vigorously. Thirty seconds' exposure to the boiling temperature deprived both the peas and the beans of their power of germination. A few of the cress-seeds exposed for this interval sprouted, but the majority were killed, and all were killed by a minute's boiling. On the other hand, a very large proportion of the mustard-seeds boiled for thirty seconds germinated. The time of exposure in the case of this seed was doubled, trebled, and quadrupled, leaving still a residue of life. The fertile mustard-seeds gradually diminished in number as the time of boiling increased, but even after two minutes' boiling many of them germinated.

And now comes a fact which I deem of some importance as regards the present inquiry. When the calico bag was abandoned, and the mustard-seeds were placed loosely in water, so as to ensure not only the free communication to them of its temperature, but free diffusion between the soluble portions of the seeds and the surrounding liquid, not one of them escaped the ordeal of thirty seconds' boiling. In the first series of experiments, the bag which held the seeds together not only exercised a protecting influence itself, but it enabled the outside seeds to act as shields to the inside ones. Assuredly in a far higher degree will cheese shield germs contained within it. Unlike fruit and meat it is highly impervious to water. It thus wards off the liquid on which the softening and swelling of the germ depend, so that within such a substance the life of a germ might be indefinitely prolonged.

* These chambers were prepared and their tubes charged prior to the introduction of hay into our laboratory last autumn, otherwise the immunity of a single one of them could not have been secured. The chambers employed had stood over from my last investigation, and no pains had been taken to render them air-tight.