Further observations on the temperature at which bacteria, vibriones, and their supposed germs are killed when immersed in fluids or exposed to heat in a moist state : and on the causes of putrefaction and fermentation / by H. Charlton Bastian, M.A.

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FURTHER OBSERVATIONS

ON THE

TEMPERATURE AT WHICH *BACTERIA, VIBRIONES,*

AND THEIR

SUPPOSED GERMS ARE KILLED

WHEN EXPOSED TO HEAT IN A MOIST STATE; AND ON THE CAUSES OF PUTREFACTION AND FERMEMTATION.

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Whilst a heat of 140° F. (60° C.) appears to be destructive to *Bacteria*, *Vibriones*, and their supposed germs in a neutral saline solution, a heat of 149° or of 158° F. is often necessary to prevent the occurrence of putrefaction in the inoculated fluids when specimens of organic infusions are employed. What is the reason of this difference? Is it owing to the fact that living organisms are enabled to withstand the destructive influence of heat better in such fluids than when immersed in neutral saline solutions? At first sight it might seem that this was the conclusion to be drawn. We must not, however, rest satisfied with mere superficial considerations.

The problem is an interesting one; yet it should be clearly understood that its solution, whatever it may be, cannot in the least affect the validity of the conclusion arrived at in my last paper, viz. that living matter is certainly capable of arising *de novo*. We were enabled to arrive at the conclusion above mentioned regarding Archebiosis by starting with the undoubted fact that a heat of 158° F. reduces to a state of potential death all the *Bacteria*, *Vibriones*, and their supposed germs which an organic infusion may contain. The inquiry upon which I now propose to enter, therefore, touching the degree of heat *below this point* which may suffice to kill such organisms and their supposed germs in an organic infusion, and touching the cause of the delayed putrefaction apt to take place in inoculated organic infusions which have been heated to temperatures above 140° and below 158° F., is one lying altogether outside the chain of fact and inference by which the occurrence of Archebiosis is proved.

It seems to me that the solution of the problems which form the subject of the present communication can only be safely attempted by keeping constantly before our minds two main considerations:—

Thus, in the experiments whose results it is now our object to endeavour to explain, the fluids have been inoculated with a compoun consisting partly (*a*) of living units, and partly (*b*) of a drop of a solution of organic matter in a state of molecular change; so that in many cases where putrefaction has been initiated after the inoculating compound has been heated to certain temperatures, there is the possibility that this process of putrefaction may have been induced (in spite of the death of the organisms and their germs) owing to the influence of *b*, the dissolved organic matter of the inoculating compound; that is to say

the heat to which the mixture has been exposed may have been adequate to kill all the living units entering into the inoculating compound, although it may not have been sufficient to prevent its not-living organic matter acting as a ferment upon the infusion*.

And there are, I think, the very best reasons for concluding that in all the cases in which turbidity has occurred after the organic mixtures have been subjected to a heat of 140° F. (60° C.) and upwards, this turbidity has been due, not to the survival of the living units, but rather to the fact that the mere dead organic matter of the inoculating compound has acted upon the more unstable organic infusions in a way which it was not able to do upon the boiled saline fluids.

In order more fully to explain the grounds upon which this conclusion is based, it will now be necessary to recast the results of the 102 inoculation experiments recorded in my last communication†. They require to be thrown into a new tabular form, in order to show how the results differed amongst themselves when organic infusions of different strengths were employed. The consideration of this aspect of the question was purposely delayed, in order to avoid the introduction of unnecessary complications into my last communication, seeing that the conclusion which I then sought to establish was in no way affected by these facts.

Neutral Hay-Infusion.

Infusion of sp. gr. 1005.				Infusion of sp. gr. 1002.		
Temperature.	Number of Expts.	Date of Turbidity, if any.	Results at the Expiration of the 8th day.	Number of Expts.	Date of Turbidity, if any.	Results at the Expiration of the 8th day.
122° F. (50° C.)	1	24 hrs.	Turbid.	.....	.....	.....
131° F.	6	48 hrs.	All turbid.	1	48 hrs.	Turbid.
140° F. (60° C.)	7	1 in 48 hrs. 6 in 60 hrs.	All turbid.	2	1 in 3 days. 1 in 8 days.	All turbid.
149° F.	.....	.....	.....	4	2 in 5 days. 1 in 8 days.	Three turbid. One clear.
158° F. (70° C.)	.....	.....	.....	15	.....	All clear.
167° F.	.....	.....	.....	4	.....	All clear.
176° F. (89° C.)	12	.....	All clear.	.....	.....	.....

* See 'The Beginnings of Life,' vol. ii. p. 2.

† See Proceedings of the Royal Society, No. 143, p. 230.

Acid Turnip-Infusion.

Infusion of sp. gr. 1008.				Infusion of sp. gr. 1005.		
Temperature.	Number of Expts.	Date of Turbidity, if any.	Results at the Expiration of the 8th day.	Number of Expts.	Date of Turbidity, if any.	Results at the Expiration of the 8th day.
122° F.	.....	.....	.....	.....	.....	.....
131° F.	5	24 hrs.	All turbid.	2	48 hrs.	All turbid.
140° F.	6	40 hrs.	All turbid.	6	4 in 3 days. 2 in 4 days.	All turbid.
149° F.	3	5 days.	All turbid.	7	1 in 3 days. 1 in 7 days. 2 in 8 days.	Four turbid. Three clear.
158° F.	.....	.....	.....	17	.....	All clear.
167° F.	.....	.....	.....	4	.....	All clear.
176° F.	.....	.....	.....	.....	.....	.....

Reference will be made to these Tables in the setting forth of my reasons for the conclusion that the more or less delayed putrefaction which takes place in inoculated organic infusions raised to the temperature of 140° F., and to other degrees of heat above this point, is due to the influence of the not-living ingredient (*b*) of the inoculating compound. These reasons are the following:—

1. Because the turbidity which has occurred in inoculated organic infusions that have been subjected to a temperature of 140° F. has always manifested itself appreciably later, and advanced much more slowly than in similar mixtures which had not been heated above 131° F.; whilst it has commenced even later, and progressed still more slowly, when occurring in mixtures previously heated to 149° F. Such facts might be accounted for by the supposition that exposure in these organic fluids to the slightly higher temperature suffices to retard the rate of growth and multiplication of the living units of the inoculating compound, although the facts are equally explicable upon the supposition that the later and less energetic putrefactions are due to the sole influence of the mere organic matter of the inoculating compound.

2. So far as the evidence embodied in the Tables goes, it tends to show that the more unstable different specimens of similar infusions are (that is, the stronger they are), the more rapidly and frequently does late turbidity ensue, and the more this late turbidity approaches, both in time of onset and in rate of increase, to that which occurs when inoculated infusions are not heated to more than 131° F.—when both living and not-living elements of the inoculating compound act conjointly as ferments. Such facts show quite clearly that where the intrinsic or predisposing causes of change are strong, there less potent exciting agencies are more readily capable of coming into play; but they still do

not enable us to decide whether the exciting cause of this delayed turbidity is in part the living element whose vitality and rate of reproduction has been lowered by the heat, or whether the effects are wholly attributable to the mere organic matter of the inoculating compound.

So far, therefore, we have concomitant variations which are equally compatible with either hypothesis. But it will be found that each of the three succeeding arguments speaks more and more plainly against the possible influence of the living element, and in favour of the action of the organic matter of the inoculating compound, as an efficient exciting cause of the delayed putrefactions occurring in the cases in question.

3. As stated in my last communication *, when single drops of slightly turbid infusions of hay or turnip previously heated to 140° F. are mounted and securely cemented as microscopical specimens, no increase of turbidity takes place, although drops of similar infusions heated only to 122° F. do notably increase in turbidity (owing to the multiplication of *Bacteria*) when mounted in a similar manner. Under such restrictive conditions as these, in fact, a drop of an inoculated and previously heated organic infusion behaves in precisely the same manner as a drop of a similarly treated ammonic-tartrate solution. In each case, when heated to 140° F., turbidity does not occur, apparently because there are no living units to multiply, and because in these mere thin films of fluid dead ferments are as incapable of operating upon the organic fluids as they are upon the ammonic-tartrate solutions.

4. Because, in the case of the inoculation of fluids which are not easily amenable to the influence of dead ferments, such as a solution containing ammonic tartrate and sodic phosphate, this delayed turbidity does not occur at all. Such inoculated fluids become rapidly turbid when heated to 131° F., though they remain clear after a brief exposure to a temperature of 140° F. When the living units in the inoculating compound are led, there is nothing left to induce turbidity in such solutions. The mere fact that these fluids do not undergo change when exposed to the air proves conclusively that they are very slightly amenable to the influence of the ordinary dead organic particles and fragments with which the atmosphere abounds. The absence of delayed turbidity in these fluids serves, therefore, to throw much light upon the cause of its occurrence in the organic infusions.

5. And, lastly, I can adduce crucial evidence supplied by the "Method of Difference," speaking with its accustomed clearness. Two portions of the same hay- or turnip-infusion can be inoculated in such a manner as to supply us with the information we require. In the one case we may employ a drop of a turbid ammonic-tartrate solution previously heated to 140° F., in which, therefore, the living units would certainly be killed; whilst in the other we may add an unheated drop of the same turbid saline solution to the organic fluid, and then heat this mixture also to

* *Loc. cit.* p. 228.

the temperature of 140° F. The comparative behaviour of these two inoculated fluids (placed, in the ordinary manner, in previously boiled corked phials) should be capable of showing us whether the living elements of the inoculating compound were able to survive when heated in the organic infusion. If they did survive, the fluids inoculated in this manner ought to undergo putrefaction earlier and more rapidly than those inoculated with the drop of turbid fluid, in which we know that the *Bacteria*, *Vibriones*, and their supposed germs would have been reduced to a state of potential death. With the view of settling this question, therefore, the following experiments were made:—

Description of Experiments.	Results.	Inferences.
A. Boiled ammonic-tartrate solution, inoculated with an unheated drop of a similar solution turbid with *Bacteria* &c.	Turbid in 40 hrs.	That boiled ammonic-tartrate solution is a fluid inoculable by living *Bacteria* &c., and favourable for their growth and rapid multiplication.
B. Boiled ammonic-tartrate solution, inoculated with a drop of a turbid saline solution previously heated to 140° F.	Clear at expiration of 8th day.	That *Bacteria*, *Vibriones*, and their supposed germs are either killed or deprived of all power of multiplication when heated to 140° F. in this fluid.
C. Boiled turnip- and hay-infusions, inoculated with a drop of a turbid saline solution previously heated to 140° F.	Turnip-infusions turbid in 2½ days. Hay-infusions clear at expiration of 8th day.	The precisely similar behaviour of the turnip- and hay-infusions of series C and series D respectively shows that the *Bacteria*, *Vibriones*, and their supposed germs are as inoperative in series D as they are known to be in series C; whilst the behaviour of the hay-infusions shows that they are little amenable to the influence of the drop of the saline fluid when its living units are killed.
D. Boiled turnip- and hay-infusions, inoculated with a drop of an unheated turbid saline solution, the inoculated fluid being subsequently heated to 140° F.	Turnip-infusions turbid in 2½ days. Hay-infusions clear at expiration of 8th day.	
E. Boiled turnip- and hay-infusions, inoculated with a drop of an unheated saline solution, the inoculated fluid being subsequently heated to 131° F.	Turnip-infusions turbid in 28 hrs. Hay-infusions turbid in 38 hrs.	Shows that a heat of 131° F. is not sufficient to kill *Bacteria*, *Vibriones*, and their supposed germs in organic infusions, and, again, that turnip-infusions are more rapidly influenced by such an inoculating agent than some hay-infusions*.

No experiments could speak more decisively. Those of series B show that *Bacteria*, *Vibriones*, and their supposed germs are either actually or potentially killed when heated to 140° F. in the neutral saline fluid,

* These experiments of series C, D, and E were many times repeated with specimens of the same turnip- and hay-infusions, the specific gravity of the former being about 1008 and that of the latter 1005. Different specimens of hay especially vary so much that it becomes absolutely essential to use portions of the same infusion for the comparative experiments of these different series.

which the experiments of series A show to be eminently favourable for their growth and reproduction. Being certain, therefore, that the living units are killed in the drops with which the fluids of series C were inoculated (because they were drops of the same fluid as was employed in series B), we may be equally certain that the turbidity and putrefaction which did ensue in the turnip-solutions of series C were due to the influence of the mere dead constituents of these drops of the turbid saline fluid; whilst, seeing that the behaviour of the fluids of series D was precisely similar to those of series C, we have a perfect right to infer that this series of fluids (D) was as devoid of living units as those of C are known to be—that is, that *Bacteria*, *Vibriones*, and their supposed germs are killed by the temperature of 140° F. in organic fluids, just as they are in saline fluids, although, as shown by the experiments of series E, they do not succumb to a heat of 131° F. These experiments of series C and D further illustrate the different degrees of amenability of different organic fluids to the same dead ferments; whilst the comparison of the results with the hay-infusions of series C and D with those previously cited (in which the inoculating compound was a drop of an organic infusion heated to the same temperature of 140° F.) will illustrate the different influence of dissimilar dead ferments upon infusions of the same kind.

The evidence now in our possession shows, therefore, that whilst the temperature at which living ferments cease to be operative varies within very narrow limits (131°–140° F.)*, that which destroys the virtues of not-living ferments varies within much wider limits, and depends not only upon the amount of heat employed, but also upon the nature of the putrescible or fermentable liquid to which such ferment is added, in conjunction with the degree of heat and other conditions to which the mixture is subsequently exposed†. Here, therefore, we have evidence as to the existence of a most important difference between living and not-living ferments, which has always been either unrecognized or more or less deliberately ignored by M. Pasteur and his followers‡. This differ-

* Liebig has proved that a temperature of 140° F. kills *Torulæ*, and always suffices to arrest a process of fermentation taking place under their influence in a sugar solution. *Torulæ* heated in water to 140° F. also fail to initiate fermentation in a sugar solution. I have also found that an exposure to a temperature of 131° F. for five minutes always suffices to destroy the life of Desmids, Euglenæ, Amœbæ, Monads, Ciliated Infusoria, Rotifers, Nematoids, and other organisms contained in specimens of pond-water. All these lower organisms seem to be destroyed at about the same temperature, as might have been expected from the fundamental relationship which must exist between these several varieties of the one substance—living matter.

† See 'The Beginnings of Life,' vol. i. p. 437.

‡ See, for instance, all M. Pasteur's celebrated experiments in which he had recourse to an "ensemencement des poussières qui existent en suspension dans l'air," as recorded in chaps. iv. & v. of his memoir in 'Ann. de Chimie et de Physique,' 1862. M. Pasteur was engaged in an investigation one of the avowed objects of which was to determine whether fermentation could or could not take place without the intervention of

ence is, moreover, thoroughly in accordance with the broad physico-chemical theory of fermentation which has been so ably expounded by Baron Liebig and others, and the truth of which may now be regarded as definitely established. According to this theory "living" matter, as a ferment, would take rank merely as a chemical compound having a tolerably definite constitution; and this, we might reasonably infer, would, like other chemical compounds, be endowed with definite properties—and amongst others that of being decomposed or radically altered by exposure to a certain amount of heat. Looked at also from this essentially chemical point of view, it would be only reasonable to expect that the molecular movements of living ferments with a lowered vitality might not be more marked or energetic than those which many not-living organic substances are apt to undergo; and this being the case, we might expect that there would often be a great practical difficulty in ascertaining whether a ferment belonging to the arbitrary and artificial (though, in a sense, justifiable and natural) category of "living" things had or had not been in operation.

It has, moreover, been most unmistakably proved that the limits of vital resistance to heat which *Bacteria*, *Vibriones*, and their supposed germs are capable of displaying are essentially the same in the three type fluids which I have employed—that is, in a weak saline fluid, in a neutral organic infusion, and in an acid organic infusion. No evidence exists really tending to show that these organisms or their germs are capable of withstanding the effects of heat better in one of such fluids than in another. We may therefore safely affirm that M. Pasteur never had any valid evidence in support of his conclusion that the germs of *Bacteria* and *Vibriones* can resist heat better in neutral or slightly alkaline solutions than in slightly acid mixtures. The experimental results which led him to arrive at such a conclusion were not logically capable of receiving any such interpretation, whilst they can be legitimately accounted for in accordance with the broader physico-chemical theory of fermentation, the truth of which has now been established*. We may also safely affirm that M. Pasteur's more specific statement, to the effect that

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living organisms, which M. Pasteur held (in opposition to many other chemists) to be the only true ferments. In his inoculating compound (dust filtered from the atmosphere), there was, as M. Pasteur was fully aware, a large amount of what his scientific opponents considered not-living ferment, whilst *possibly* there existed a certain number of living ferments. In explaining the results of his experiments, however, M. Pasteur and others thought he was pursuing a logical and scientific method when he attributed these results to the action of the possibly existing element of the inoculating compound, whilst he ignored altogether the other element which was certainly present in comparatively large quantity, and the testing of whose efficacy was the ostensible object of his research.

* I attempted to show, nearly three years ago (see 'Nature,' July 14, 1870, pp. 224–228), that the differences which M. Pasteur ascribed to differences of vital resistance of organisms in particular fluids were just as explicable in accordance with the physico-chemical theory of fermentation, by reference to the different degrees of fermentability of the several fluids.

the germs of some *Bacteria* and *Vibriones* are capable of resisting the influence of a heat of 212° F. when in the moist state, though they are killed by a temperature of 230° F., was a conclusion altogether unwarranted by the evidence which he adduced. Finding that certain fluids treated after the manner introduced by Schwann always remained quite devoid of living organisms, M. Pasteur very legitimately concluded that preexisting organisms and germs had been killed during the boiling of the liquid; but finding that when a little powdered chalk was added to fluids of the same kind (which in all other respects were treated in a similar manner) living organisms were after a time invariably found to appear, although they as invariably failed to appear when the same fluids were heated to a temperature of 230° F. (110° C.), two equally legitimate provisional conclusions were open to M. Pasteur in explanation of these facts. What did M. Pasteur do? Following the same method as he had formerly employed*, he again ignored one of the equally possible interpretations, and unsuccessfully attempted to prove, by a repetition of similar reasoning†, that the different results in the two series of experiments were due to the fact that the germs of *Bacteria* and *Vibriones* which had been killed by the temperature of 212° F. in the first series, were not killed by this temperature in the second series (in which a slightly alkaline fluid had been employed), although they were destroyed by the higher temperature of 230° F. Thus results which were due to the action of not-living ferments were ascribed to living ferments, and the possible action of not-living ferments was ignored, although, as I have said before, the ostensible object of M. Pasteur's researches was to inquire into the relative importance of not-living and living ferments, or whether, in fact, "dead" substances (in the ordinary acceptation of the word) could act as ferments.

When viewed from the stand-point of the physico-chemical theory of fermentation, the apparently contradictory results arrived at by the same experimenter at different times or by different experimenters, in this line of research, cease to be the inexplicable puzzle which they must always appear to those who place implicit faith in the narrower and too exclusive "vital" theory of fermentation advocated by M. Pasteur and his followers.

My investigations have convinced me that, with regard to degree of fermentability, the various fermentable fluids and mixtures are divisible into three distinct subclasses:—

I. There are what may be called self-fermentable fluids or mixtures—that is, fluids or mixtures which, after exposure to a temperature of 212° F. or higher, are still capable of undergoing fermentative changes without the addition of less-heated matter, either not-living or living. The changes occurring in these self-fermentable fluids (in which preexisting living things have been killed), when strictly protected from contact with adventitious particles, vary in rapidity and in intensity from the

* See note ‡ on page 330.

† See Ann. de Chim. et de Phys. 1862, pp. 60–65.

highest to the very lowest degrees of fermentability. These gradations are dependent principally upon the nature of the fluids or mixtures employed, and upon the degree of heat to which they have been submitted, though partly also to the temperature, pressure, presence or absence of filtered air, and degree of light to which the mixtures are subsequently exposed. For the sake of convenience, these gradations may be ranged into several distinct groups, though of course any such divisions as I am now about to sketch are purely artificial and are connected with one another in nature by innumerable transitions.

Nature of Fluids.	Nature of Results.
A. Turnip-infusion with cheese, turnip-infusion neutralized by liquor potassæ, ordinary turnip-infusion, strong hay-infusion, &c.	Within two to four days marked turbidity, owing to the appearance of swarms of *Bacteria* and *Vibriones*. Fluids more or less fœtid. (*Putrefaction.*)
B. Turnip-infusion neutralized by liquor potassæ, ordinary turnip-infusion, ordinary hay-infusion, &c.	No uniform turbidity, but growth of flocculi in a more or less clear liquid. After a time the flocculi (composed of aggregated *Bacteria* and *Vibriones*) gradually subside, and the activity of the process ceases. Fluids either fœtid or having a mere sour odour.
C. Beer-wort*, &c.	Fluids which become more or less uniformly and rapidly turbid, owing to the appearance of swarms of *Torulæ*.
D. Weak hay-infusions, urine, solutions containing ammonic carbonate and sodic phosphate with minute organic impurities, &c.	Do not become visibly turbid or produce visible flocculi, although on microscopical examination they may be found to contain living *Bacteria* pretty uniformly distributed, but in comparatively small quantities. The odour is often not more appreciably altered than the clearness of such solutions.
E. Weak hay-infusions, urine, solutions containing ammonio-citrate of iron and minute organic impurities†, &c.	Same as in the last group‡, though after weeks or months a dirty-looking sedimentary matter slowly accumulates at the bottom of the flask, which on microscopical examination is found to be composed partly of *Bacteria* with *Vibriones* and *Leptothrix*, and partly of *Torulæ* or more thick-walled fungus-germs.
F. Solutions of ammonic tartrate and sodic phosphate with minute organic impurities, &c.	Same as in the last group, only the dirty sedimentary matter which accumulates never contains either *Bacteria*, *Vibriones*, or *Leptothrix*. Living *Torulæ* and thick-walled fungus-germs in various stages of formation are frequently met with, and also, occasionally, a mycelium resulting from the development of some of these bodies.

* I have had no experience with such a fluid myself. M. Pouchet's observations were, however, most striking on this subject (see his 'Nouvelles Experiences,' Paris, 1864, p. 190).

† In solutions containing iron, green organisms may subsequently be found (see 'Beginnings of Life,' vol. ii. p. 157).

‡ This, in fact, is in many cases the kind of change which the fluids last described ultimately undergo.

Nature of Fluids.	Nature of Results.
G. Weak or strongly acid infusions, and also many saline solutions containing organic impurities.	May remain permanently barren, and never show any traces of organisms, either dead or living *.

II. To the second subclass belong fluids which, after exposure to a temperature of 212° F. or higher, may be kept clear or apparently unaltered so long as they are shut off from contact with unheated atmospheric or other organic particles, but which do undergo putrefaction, or more or less marked fermentation, soon after they are brought into contact even with mere not-living organic matter.

The experiments recorded in this communication have most conclusively proved the efficacy of not-living organic matter as a ferment or inciter of change in previously barren fluids. And combining the knowledge derived from these experiments with that which we now possess concerning the absence of living *Bacteria*, *Vibriones*, and their germs in the air, together with the known prevalence of minute organic particles and fragments of various kinds, the explanation of M. Pasteur's celebrated experiments in which he had recourse to an "ensemencement des poussières qui existent en suspension dans l'air," becomes quite easy and legitimate without having recourse to the hypothesis of Panspermism †. Now, also, are we enabled to understand all the apparent inconsistencies of those experiments in which previously boiled fluids have been exposed to the ordinary air of different localities, and have then been resealed. If many specimens of these fluids remained unchanged, whilst others, after a few days, swarmed with *Bacteria* and *Vibriones*, we may now very safely attribute these previously puzzling results to the comparative absence or presence of organic fragments in the particular volumes of air which chanced to get into the flasks, and to the different nature of the fluids employed by different experimenters ‡.

* See many negative experiments recorded in 'The Beginnings of Life,' vol. i. ch. xi. Mr. W. N. Hartley has laboured very industriously to disprove something which I never asserted (see Proceedings of Royal Society, vol. xx. p. 140). In my early paper in 'Nature' I expressly stated that organic impurities were always present in the saline solutions which I employed; and, as may be seen by the note appended to the conclusions of that paper, I never claimed to have established that living organisms could appear in saline solutions free from traces of organic impurity. Mr. Hartley did attempt to work with approximately pure saline solutions, and in other respects also the conditions of his experiments differed so much from mine, that the results which he obtained could not possibly be considered to disprove what I had previously stated. Some of his flasks were heated to 180° C., a temperature about which I had said nothing; and whilst his organic infusions were too weak, some of his saline solutions were too concentrated, though the strengths of others were not given at all. Fluids were also employed (such as urine, heated to 130° C.) which I had not made use of, and which I should not have thought of experimenting with.

† See the experiments before alluded to, which are recorded in chaps. iv. & v. of his Memoir.

‡ See M. Pasteur's Memoir, chap. vii., and also Compt. Rend. Nov. 5, 1860. See

Many of the fluids which habitually remain clear after a previous ebullition in flasks whose necks have been plugged with cotton-wool, many times bent, or hermetically sealed after the entry of calcined air, or when enclosed in vessels which are completely full (in Gruithuisen's fashion), belong to this subclass. In other cases, however (as in many of those instances where urine or hay- or turnip-infusions have been employed), those who do not content themselves with a mere naked-eye inspection of the apparently pure fluids would find on microscopical examination of the sediment that such fluids were to a low degree self-fermentable—that they correspond, in fact, with group E of the last subclass*; whilst, in addition, my researches have shown that many of such fluids are capable of being rendered self-fermentable to a marked degree, if, instead of subjecting them to contact with calcined air or variously filtered air, its reflux after ebullition is altogether prevented by hermetically sealing the neck of the flask during ebullition. Operating in this way, I have repeatedly found that fluids freed from the pressure of air and from its influence altogether become to a marked extent self-fermentable, although the same fluids exposed to filtered or calcined air under ordinary atmospheric pressure remain unaltered and barren, or at most exhibit the very low degree of fermentability referred to as characterizing group E†. But just as amongst the self-fermentable fluids we find there are some which only engender *Torulæ* or other allied fungus-germs, so now we find that some previously boiled fluids, even when fully exposed to the air, swarm only with *Torulæ*. Those exciting agents derived from the atmosphere which, with one set of fluids, initiate changes leading to the evolution of *Bacteria*, with another set lead only to the evolution of *Torulæ*. And whilst telling us that the *Bacteria* which appear in previously barren fluids after exposure to air are not due to their contamination with germs of *Bacteria*, some observers would have us conclude that the *Torulæ* which appear in other previously barren fluids after a similar exposure are the products of preexisting aërial germs of such organisms. This conclusion, however, cannot readily be accepted in the face of the evidence derived from the closed-flask experiments with self-fermentable fluids of the lowest degree‡. Such experiments, in fact, render the hypothesis as

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also a record of other experiments made with the air of alpine regions by MM. Pouchet, Joly, and Musset, in Compt. Rend. Sept. 21, 1863.

* Other fluids richer in organic matter or otherwise more favourably endowed, instead of presenting this low degree of self-fermentability, are notably prone to undergo change when we attempt to preserve them in the manner described, although such modes of preparation do suffice for preserving so many fluids. This has been fully admitted by Schrœder and Dusch, Schwann, Pasteur, and others.

† In illustration of this statement see 'The Beginnings of Life,' Appendix C, Exps. viii., ix., xiv., xv., xviii., xx., xxvi., xxx., xxxiii., and xxxvi.

‡ The only evidence in favour of such a conclusion is not one jot more conclusive than that which was formerly adduced in favour of the universal prevalence of *Bacteria*-germs in the air.

to the widespread distribution of aërial germs of *Torulæ* wholly unnecessary, by showing that certain fluids, by reason of certain intrinsic peculiarities, when they undergo fermentation give rise to *Torulæ* only. We are thus led to conclude that whilst some fluids are capable of engendering both kinds of organisms, others tend only to produce one or other of them—whether the fluids are contained in closed flasks or in open vessels exposed to the incidence of atmospheric particles. I have more than once seen nothing but *Torulæ* appear in an infusion of turnip exposed to the air after it had been heated in a closed tube to a temperature of 293° F. for twenty minutes, and I have once seen the same thing occur in an unheated infusion of turnip exposed to the air, though on all other occasions such infusions have swarmed only with *Bacteria* and *Vibriones*. On the other hand, a boiled ammonic-tartrate solution exposed to the air, though protected from an excess of atmospheric particles (for the advent of a large number of these might in some cases incite putrefaction), is never found to contain *Bacteria*; the fluid continues clear, though a sediment gradually accumulates at the bottom of the flask, amongst which *Torulæ* and other fungus-germs are constantly to be found—more numerous though otherwise very similar to those which are to be met with in flasks closed during ebullition, or in others to which only filtered air is admitted. Although *Torulæ* only appear in such fluids, they continue all the time to be eminently inoculable by *Bacteria*; and, again, when the *Torulæ* begin to decay they are apt to incite a more or less manifest putrefaction, during which the fluid gradually becomes turbid with *Bacteria*. It is, in fact, a general rule that putrefaction is apt to supervene upon a fermentation of a more smouldering type.

III. In the third subclass I include fluids which, after exposure to 212° F. or higher temperatures, are unable, either alone or under the influence of ordinary atmospheric particles or fragments, to undergo putrefaction, although such a process can invariably be initiated by bringing the fluids into contact with living ferments. As examples of such fluids, I may cite the neutral saline solution to which I have so often referred and that known as Pasteur's solution. Other fluids of the same kind have lately been referred to by Professor Huizinga*. The fact that certain fluids cannot be made to undergo putrefaction by the influence of dead organic particles, although they become at once amenable to the influence of living units, unmistakably shows the superior potency of living ferments; their action has, moreover, invariably proved to be certain and inevitable in all the cases in which they were known to be present. Even these least fermentable fluids of our third subclass invariably become turbid within three days after inoculation with living units, if maintained at a temperature of about 70° F.; whilst when other more changeable fluids are inoculated, putrefaction ensues with equal certainty, though with much greater rapidity.

* See 'Nature,' March 20th, 1873, p. 380.

What we have learned, therefore, concerning the *invariable uniformity* of simple inoculation experiments should of itself teach us how difficult it would be to account for cases of delayed putrefaction, or for cases in which a mere smouldering fermentation is set up, by the old though now well-nigh exploded notion of contamination by preexisting germs. Where living ferments really exist, the course of events is definite and almost invariable in its rapidity; but where fermentation takes place as a result of chemical changes occurring in the fluid itself (either by its own unaided powers, or under the stimulating influence of a less-heated organic ferment) there is abundant room for all the irregularity and variation actually encountered. These cases of irregularity and variation have always, on other grounds, defied all legitimate attempts to bring them individually within the pale of a narrow and exclusively "vital" theory of fermentation; and now a wider experience with living ferments equally tends to show the impossibility of legitimately explaining a great mass of irregular phenomena by means of agents whose action is shown to be constant and almost invariable.

Thus it can now be proved, by evidence of a most unmistakable nature, that the process of putrefaction which invariably occurs in previously boiled putrescible infusions contained in flasks with narrow but open necks is not commonly (is, perhaps, only very rarely) initiated by living germs or organisms derived from the atmosphere; it can also be proved that putrefaction and the appearance of swarms of living organisms may occur in some boiled fluids when they are simply exposed to air which has been filtered through a firm plug of cotton-wool or through the narrow and bent neck of a flask, to air whose particles have been destroyed by heat, or even in fluids hermetically sealed in flasks from which all air has been expelled. The evidence in our possession is therefore most complete on this part of the subject: it shows beyond all doubt, not only that putrefaction may and does very frequently occur under conditions in which the advent of atmospheric particles, whether living or dead, is no longer possible, but also that living particles derived from the atmosphere can only be very rare and altogether exceptional initiators of the putrefaction which invariably occurs in previously boiled infusions exposed to the air.

Again, the evidence which we now possess with reference to the influence of heat upon *Bacteria*, *Vibriones*, and their supposed germs is no less decisive. It has been unmistakably proved that such organisms and their imaginary germs are either actually or potentially killed by a brief exposure to the temperature of 140° F. when in the moist state; and it had also been previously established that they are invariably killed by desiccation even at much lower temperatures*.

* See the experiments and conclusions of Dr. Burdon Sanderson in Thirteenth Report of Med. Officer of Privy Council, p. 61. This fact of the inability of these or-

But if living germs do not come from the air to contaminate the previously boiled fluids, and if it is not possible for any of them to have escaped the destructive influence of heat in the boiling fluid or on the walls of the vessel in which the fluid is contained, what can be the mode of origin of the swarms of living things which so rapidly and invariably appear in such infusions when contained in open flasks, and which so frequently appear when the infusions are contained in flasks whose necks are closed against atmospheric particles of all kinds? They can only have arisen by the process which I have termed Archebiosis.

Conclusions.

If a previously boiled ammonic-tartrate solution remains free from *Bacteria* and *Vibriones* when exposed to the air, it is because the air does not contain living organisms of this kind or their supposed germs, and because mere dead organic particles are not capable of initiating putrefaction in such a fluid.

And if ordinary organic infusions previously boiled and exposed to the air do rapidly putrefy, though *some* of the same infusions when exposed only to filtered air remain pure, it is because such fluids are, in the absence of living units, quite amenable to the influence of the dead organic particles which the air so abundantly contains, although they are not self-fermentable.

Whilst if other more changeable fluids, after previous boiling, when exposed to filtered air or cut off altogether from contact with air, do nevertheless undergo putrefaction or fermentation, it is because these fluids are self-fermentable, and need neither living units nor dead organic particles to initiate those putrefactive or fermentative changes which lead to the evolution of living organisms.

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ganisms and their germs to resist desiccation shows the futility of some objections which have been from time to time raised by those who thought that *Bacteria*, *Vibriones*, and their germs might resist the destructive influence of heat by adhesion to the glass above the level of the fluid, or even in the fluid itself, just as dried and very thick-coated seeds have been known to do. Dry heat would seem to be even more fatal to such organisms and their germs than a moist heat of the same degree, owing to their extreme inability to resist desiccation: if they become dry they are killed at a temperature of about 104° F., whilst if they remain moist they succumb, as we have seen, to a temperature of 140° F.