

- II. "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." By H. CHARLTON BASTIAN, M.A., M.D., F.R.S., Professor of Pathological Anatomy in University College, London. Received March 4, 1873.

For more reasons than one we may, perhaps, now look back with advantage upon the friendly controversy carried on rather more than a century ago between the learned and generous Abbé Spallanzani and our no less distinguished countryman Turberville Needham. Writing concerning his own relation to Needham, the Abbé said\*, "I wish to deserve his esteem whilst combating his opinion"; and, in accordance with this sentiment, we find him treating his adversary's views with great respect, and at the same time repudiating much of the empty and idle criticism in which so many of Needham's contemporaries indulged with regard to his work. This criticism, Spallanzani says†, "Without looking into details, contented itself by throwing doubt upon some of the facts, and by explaining after its own fashion others whose possibility it was willing to admit." He moreover warmly reprobated the ignorant and disrespectful statements made by an anonymous writer who had shown himself little worthy of being heard upon the subjects in dispute. Spallanzani on this occasion very wisely said ‡:—"When it is a question concerning observations and experiments, it is necessary to have repeated them with much circumspection before venturing to pronounce that they are doubtful or untrustworthy. He who will allow himself to speak of them with contempt, and who can only attempt to refute them with writings composed by the glimmer derived from a treacherous lamp, will not find himself in a condition to retain the esteem of learned men." The anonymous writer (in his '*Lettres à un Américain*'), to whom Spallanzani referred, had gone so far as to doubt the statements of Needham as to the constant appearance of organisms in infusions which had been previously boiled, and also intimated that even if they were to be found, it was only because they had been enabled to resist the destructive influence of the boiling fluid. This latter assertion was emphatically denied by Spallanzani, his denial being based upon a most extensive series of experiments with eggs in great variety and with seeds of all degrees of hardness; these were all found to be killed by a very short contact with boiling water. Spallanzani had thoroughly satisfied himself that even very thick-coated seeds could not resist this destructive agent; whilst he thought that the

\* *Nouvelles Recherches sur les Découvertes Microscopiques et la Génération des Corps Organisés*, &c. London and Paris, 1769, vol. i. p. 69.

† *Loc. cit.* p. 9.

‡ *Loc. cit.* p. 114.

idea, entertained by some, of the eggs of the lowest infusoria being protected from the injurious influence of the boiling water by reason of their extreme minuteness, was a supposition so improbable as scarcely to deserve serious consideration. Such a notion was, he thought, wholly opposed to what was known concerning the transmission of heat. Whilst, therefore, the opinion of those who believe that eggs have the power of resisting the destructive influence of boiling water could be fully refuted, Spallanzani thought it by no means followed that the infusoria which always, after a very short time, appeared in boiling infusions had arisen independently of the existence of eggs. The infusions being freely exposed to the air, it was very possible that this air had introduced eggs into the fluids, which by their development had given birth to the infusoria\*.

After the lapse of a century it has at last been clearly shown that this supposition of aerial contamination advanced by Spallanzani (warrantable and natural as it was at the time) is one which, in the great majority of cases, is devoid of all foundation in fact, so far as concerns the organisms essentially associated with processes of putrefaction, viz. *Bacteria* and *Vibriones*. The means of proving this statement, based upon independent observations made by Professor Burdon Sanderson and myself, were recently submitted to the consideration of the Royal Society†. Before the reading of this communication I was under the impression that almost every one of those who had taken part in the controversies which had been carried on both here and abroad concerning the Origin of Life were prepared to admit, as Spallanzani had done, that the eggs or germs of such organisms as appear in infusions were unable to survive when the infusions containing them were raised to the temperature at which water boils. This impression was produced in part by the explicit statements on this subject that had been made by very many biologists, and also in part by a comparatively recent and authoritative confirmation which this view as to the destructive effects of boiling infusions upon *Bacteria* had received. Little more than two years ago Professor Huxley, as President of the British Association for the Advancement of Science, recorded experiments in his Inaugural Address which were obviously based upon this belief as a starting-point; and subsequently, in one of the Sectional Meetings, after referring to some of my experiments, and to the fact that all unmistakably vital movements ceased after *Bacteria* had been boiled, Professor Huxley added‡:—"I cannot be certain about other persons, but I am of opinion that

\* A few pages further on this view is thus shortly expressed:—"Il est évident que toutes les tentatives faites avec le feu, peuvent bien servir à prouver que les animaux microscopiques ne naissent point des œufs que l'on supposait exister dans les infusions avant qu'on leur fit sentir le feu; mais cela n'empêche pas qu'ils n'aient pu être formés de ceux qui auront été portés dans les vases après l'ébullition."

† See Proceedings of Royal Society, No. 141, 1873, p. 129.

‡ See Report in Quart. Journ. of Microscop. Science, Oct. 1870.

observers who have supposed they have found *Bacteria* surviving after boiling have made the mistake which I should have done at one time, and, in fact, have confused the Brownian movements with *true living* movements." Some eminent biologists do not now (in reference to the experiments cited in my last communication) suggest that the organisms found in the infusions were dead and had been there before the fluids were boiled: they express doubts concerning that which seems formerly to have been regarded as established, and now wish for evidence to show that the germs of *Bacteria* and *Vibriones* are killed in a boiling infusion of hay or turnip, as they have been proved to be in "Pasteur's Solution" and in solutions containing ammoniac tartrate and sodic phosphate.

With the view of removing this last source of doubt more effectually, and also of refuting the unwarrantable\* conclusion of M. Pasteur, to the effect that the germs of *Bacteria* and *Vibriones* are not killed in neutral or slightly alkaline fluids at a temperature of 212° F., I almost immediately after the reading of my last communication commenced a fresh series of experiments.

Nearly two years ago, in my 'Modes of Origin of Lowest Organisms,' I brought forward evidence to show that *Bacteria*, *Vibriones*, and their supposed germs are killed at a temperature of 140° F. (60° C.) in neutral or very faintly acid solutions containing ammoniac tartrate and sodic phosphate, and also evidence tending to show that these living units were killed in neutral infusions of hay and in acid infusions of turnip at the same temperature.

The crucial evidence adduced concerning the degree of heat destructive to *Bacteria*, *Vibriones*, and their germs, in the saline solution, was of this nature. The solution had been shown to be incapable of engendering *Bacteria* and *Vibriones* (under all ordinary conditions) after it had been boiled, although it still continued capable of supporting the life and encouraging the rapid multiplication of any of these organisms which were purposely added to it. Some of this boiled solution, therefore, was introduced into flasks previously washed with boiling water; and when the fluids had sufficiently cooled, that of each flask was inoculated with living *Bacteria* and *Vibriones*—in the proportion of one drop of a fluid quite turbid with these organisms to one fluid ounce of the clear saline solution†. These mixtures containing an abundance of living organisms were then heated to various temperatures, ranging from 122° F. (50° C.) to 167° F. (75° C.); and it was invariably found that those which had been

\* Reasons for this opinion have been fully set forth in 'The Beginnings of Life,' vol. i. pp. 374 *et seq.*; or the discriminating reader may at once find my justification for this expression by reading pp. 58–66 of M. Pasteur's memoir in 'Ann. de Chim. et de Physique,' 1862.

† Fuller details concerning these experiments may be found in the little work already mentioned at pp. 51–56, and also in 'The Beginnings of Life,' vol. i. pp. 325–332.

heated to 122° or 131° F. became quite turbid in about two days, whilst those which had been raised to 140° F. or upwards as invariably remained clear and unaltered. The turbidity in the first series having been ascertained to be due to the enormous multiplication of *Bacteria* and *Vibriones*, and it being a well-established fact that such organisms when undoubtedly living always rapidly multiply in these fluids, the conclusion seemed almost inevitable that the organisms and their germs must have been killed in the flasks which were briefly subjected to the temperature of 140° F. How else are we to account for the fact that these fluids remained quite unaltered although living organisms were added to them in the same proportion as they had been to those less-heated fluids which had so rapidly become turbid? Even if there does remain the mere possibility that the organisms and their supposed germs had not actually been killed, they were certainly so far damaged as to be unable to manifest any vital characteristics. The heat had, at all events, deprived them of their powers of growth and multiplication; and these gone, so little of what we are accustomed to call "life" could remain, that practically they might well be considered dead. And, as I shall subsequently show, the production of this potential death by the temperature of 140° F. enables us to draw just the same conclusions from other experiments, as if such a temperature had produced a demonstrably actual death\*. Seeing also that these saline solutions were inoculated with a fluid in which *Bacteria* and *Vibriones* were multiplying rapidly, we had a right to infer that they were multiplying in their accustomed manner, "as much by the known method of fission, as by any unknown and assumed method of reproduction." So that, as I at the time said†, "These experiments seem to show, therefore, that even if *Bacteria* do multiply by means of invisible gemmules, as well as by the known process of fission, such invisible particles possess no higher power of resisting the destructive influence of heat than the parent *Bacteria* themselves possess."

This is, in fact, by far the most satisfactory kind of evidence that can be produced concerning the powers of resisting heat enjoyed by *Bacteria* and *Vibriones*, because it also fully meets the hypothesis as to their possible multiplication by invisible gemmules possessed of a greater power of resisting heat, and because no mere inspection by the microscope of dead *Bacteria* can entitle us positively to affirm that they are dead, even though all characteristically vital or "true living" movements may be absent.

Facts of a very similar nature were mentioned in the same work strongly tending to show that *Bacteria* and *Vibriones* are also killed at the same temperature in other fluids, such as infusions of hay or turnip. These facts were referred to in the following statement‡:—"Thus, if on

\* See p. 232.

† Modes of Origin of Lowest Organisms, 1871, p. 60.

‡ *Loc. cit.* p. 60.

the same slip, though under different covering-glasses, specimens of a hay-infusion turbid with *Bacteria* are mounted, (a) without being heated, (b) after the fluid has been raised to 122° F. for ten minutes, and (c) after the fluid has been heated to 140° F. for ten minutes, it will be found that in the course of a few days the *Bacteria* under a and b have notably increased in quantity, whilst those under c do not become more numerous, however long the slide is kept. Facts of the same kind are observable if a turnip-infusion containing living *Bacteria* is experimented with; and the phenomena are in no way different if a solution of ammoniac tartrate and sodic phosphate (containing *Bacteria*) be employed instead of one of these vegetable infusions. The multiplication of the *Bacteria* beneath the covering-glass, when it occurs, is soon rendered obvious, even to the naked eye, by the increasing cloudiness of the film."

The facts just cited concerning the behaviour of thin films of turbid infusions which had been heated to different temperatures gave me the clue as to the proper direction of future work. It would seem that, when mounted in the manner described, such thin films of infusion continue capable of supporting and favouring the multiplication of any already existing *Bacteria* and *Vibriones*, although under such conditions no new birth of living particles appears to take place even in these fluids. The question then arose as to whether, by subjecting larger quantities of the same infusions to any particular sets of conditions, we could ensure that they also should continue to manifest the same properties; because if so, it would be almost as easy to determine the death-point of *Bacteria* and *Vibriones* when exposed to heat in these infusions, as it had been to determine it for the saline solutions already mentioned.

It was pointed out by Gruithuisen early in the present century, that many infusions, otherwise very productive, ceased to be so when they were poured into a glass vessel whilst boiling, and when this was filled so that the tightly fitting stopper touched the fluid. Having myself proved the truth of this assertion for hay-infusion, it seemed likely that, by having recourse to a method of this kind, I should be able to lower the virtues of boiled hay- and turnip-infusions to the level of those possessed by the boiled saline solution with which I had previously experimented—that is, to reduce them to a state in which, whilst they appear (under these conditions) quite unable of themselves to engender *Bacteria* or *Vibriones*, they continue well capable of favouring the rapid multiplication of such organisms.

This was found to be the case; and I have accordingly performed upwards of one hundred experiments with inoculated portions of these two infusions raised to different temperatures. The mode in which the experiments were conducted was as follows:—

Infusions of hay and turnip of slightly different strengths were employed. These infusions, having been first loosely strained through

muslin, were boiled for about ten or fifteen minutes, and then whilst boiling strained through ordinary Swedish filtering-paper into a glass beaker which had previously been well rinsed with boiling water. A number of glass bottles or tubes were also prepared, which, together with their stoppers or corks, had been boiled in ordinary tap water for a few minutes\*. They were taken out full of the boiling fluid; and the stoppers or corks being at once inserted, the vessels and their contents were set aside to cool. When the filtered infusion of hay or turnip had been rapidly cooled down to about 110° F. (by letting the beaker containing it stand in a large basin of cold water), it was inoculated with some of a turbid infusion of hay swarming with active *Bacteria* and *Vibriones*—in the proportion of one drop of the turbid fluid to each fluid ounce of the now clear filtered infusion†. The beaker was then placed upon a sand-bath, and its contained fluid (in which a thermometer was immersed) gradually raised to the required temperature. The fluid was maintained at the same temperature for five minutes by alternately raising the beaker from and replacing it upon the sand-bath. The bottles to be used were then one by one uncorked, emptied, and refilled to the brim with the heated inoculated fluid‡. The corks or stoppers were at once very tightly pressed down, so as to leave no air between them and the surface of the fluids. The beaker was then replaced upon the sand-bath and the gas turned on more fully, in order that the experimental fluid might be rapidly raised to a temperature 9° F. (5° C.) higher than it had been before. After five minutes' exposure to this temperature other bottles were filled in the same manner, and so on for the various temperatures the influence of which it was desired to test.

Thus prepared, the bottles and tubes have been exposed during the day to a temperature ranging from 65° to 75° F. And generally one had not to wait long in order to ascertain what the results were to be. In some cases, if the contents of the vessels were to become turbid, this was more or less manifest after an interval of forty-eight hours; in other cases, however, the turbidity manifested itself three or more days later: the reason of this difference will be fully discussed in a subsequent communication.

For the sake of simplicity and brevity, the necessary particulars concerning the 102 experiments have been embodied in the following Table:—

\* The vessels employed have varied in capacity from two drachms to four ounces; some have been provided with glass stoppers and others with very tightly fitting corks; and the latter I find have answered quite as well as the former. On the whole I have found tightly corked one-ounce phials to be about the most convenient vessels to employ in these inoculation experiments.

† It was found desirable to filter the infusions after they had been boiled, because the boiling generally somewhat impaired their clearness.

‡ At this stage, of course, *very great care* is needed in order to avoid all chance of accidental contamination either with living organisms or with unheated fragments or particles of organic matter.

Inoculation Experiments made with the view of ascertaining the Temperatures at which *Bacteria*, *Vibriones*, and their supposed Germs are killed in Organic Infusions.

NEUTRAL HAY-INFUSION.			
Temp. to which exposed.	Number of experiments made.	Date of Turbidity, if any.	Results at Expiration of the 8th day.
122° F. } (50° C.) } 131° F.	1 7	24 hours. 48 hours.	Turbid. All turbid.
140° F.	9	{ 1 in 48 hours. 6 in 60 hours. 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.	15	.....	All clear.
167° F.	4	.....	All clear.
176° F. } (80° C.) }	12	.....	All clear.

ACID TURNIP-INFUSION.			
Temp. to which exposed.	Number of experiments made.	Date of Turbidity, if any.	Results at Expiration of the 8th day.
122° F.	....	.....	.....
131° F.	7	{ 5 in 24 hours. 2 in 48 hours.	All turbid.
140° F.	12	{ 6 in 40 hours. 4 in 3 days. 2 in 4 days.	All turbid.
149° F.	10	{ 1 in 3 days. 3 in 5 days. 1 in 7 days. 2 in 8 days.	{ Seven turbid. Three clear.
158° F.	17	.....	All clear.
167° F.	4	.....	All clear.
176° F.	....	.....	.....

The experimental results above tabulated seem naturally divisible into three groups. Thus, when heated only to 131° F., all the infusions became turbid within two days, just as the inoculated saline solutions had done\*. Heated to 158° F. all the inoculated organic infusions remained clear, as had been the case with the saline solutions in my previous experiments when heated to 140° F. There remains, therefore, an intermediate heat zone (ranging from a little below 140° to a little below 158° F.) after an exposure to which the inoculated organic infusions are apt to become more slowly turbid, although inoculated saline solutions raised to the same temperatures invariably remain unaltered. The full explanation of these apparent anomalies I propose to make the subject of a future communication to the Royal Society; meanwhile we may quite safely conclude that *Bacteria*, *Vibriones*, and their supposed germs are either actually killed or else completely deprived of their powers of multiplication after a brief exposure to the temperature of 158° F. (70° C.).

This evidence now in our possession as to the limits of "vital resistance" to heat displayed by *Bacteria*, *Vibriones*, and their supposed germs in neutral saline solutions, and in neutral or acid organic infusions, is most pertinent and valuable when considered in relation to that supplied by other sets of experiments bearing upon the all-important problem of the Origin of Life. These latter experiments alone may possibly leave doubt in many minds; but the more thoroughly they are considered in relation to the evidence brought forward in this communication, the more fully, I venture to think, will every lingering doubt as to the proper conclusion to be arrived at be dispelled.

Thus we now know 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; but, placed under slightly different conditions such as were employed in the inoculation experiments above quoted, although infusions of the same nature do not undergo "spontaneous" putrefactive changes, yet when living *Bacteria* and *Vibriones* are added, and not subsequently heated, putrefaction *invariably* takes place and the fluids thus situated rapidly become turbid. There is therefore nothing in the conditions themselves tending to hinder the process of putrefaction, so long as living units are there to initiate it. Our experiments now show that as long as the added *Bacteria*, *Vibriones*, and their supposed germs are subjected to a heat not exceeding 131° F. (55° C.), putrefaction invariably occurs within two days; whilst, on the contrary, whenever they are subjected to a temperature of 158° F. (70° C.) putrefaction does not occur. To what can this difference be due, except to the fact that the previously living organisms, which, when living, always excite putrefaction, have been

\* In the experiments already referred to.



killed by the temperature of 158° F.? It would be of no avail to suppose that the absence of putrefaction in these latter cases is due to the fact that a heat of 158° F., instead of killing the organisms and their germs, merely annuls their powers of reproduction, because in the other series of experiments (with which these have to be compared), where similar fluids are exposed to ordinary or purified air, or are shut off from the influence of air altogether, the most active putrefaction and multiplication of organisms takes place in two, three, or four days, in spite of the much more potent heat of 212° F. to which any preexisting germs or organisms must have been subjected. The supposition, therefore, that the *Bacteria*, *Vibriones*, and their germs were not killed in our inoculation experiments at the temperature of 158° F., but were merely deprived of their powers of reproduction, would be no gain to those who desire to stave off the admission that *Bacteria* and *Vibriones* can be proved to arise *de novo* in certain cases. Let us assume this (which is indisputably proved by these inoculation experiments), viz. that an exposure to a temperature of 158° F. (70° C.) for five minutes deprives *Bacteria*, *Vibriones*, and their germs of their usual powers of growth and reproduction—that is, that it reduces them to a state of potential, if not necessarily to one of actual death. What end would be served by such a reservation? The impending conclusion could not be staved off by means of it. The explanation of what occurs in the other set of experiments, where the much more potent heat of 212° F. is employed, still would not be possible without having recourse to the supposition of a *de novo* origination of living units, so long as those which may have pre-existed in the flasks could be proved to have been reduced to such a state of potential death. It would be preposterous, and contrary to the whole order of Nature, to assume that the vastly increased destructive influence of a heat of 212° F. had restored vital properties which a lesser amount (158° F.) of the same influence had completely annulled.

The evidence supplied by these different series of experiments, in whichever way it is regarded, as it seems to me, absolutely compels the logical reasoner to conclude that the swarms of living organisms which so often make their appearance in boiled infusions treated in one or other of the various modes already proved to be either destructive or exclusive of preexisting living things are the products of a new brood of “living” particles, which, in the absence of any coexisting living organisms, must have taken origin in the fluid itself. For this mode of origin of living units, so long spoken of and repudiated as “spontaneous generation,” I have proposed the new term Archebiosis.