CHAPTER IX.

SPONTANEOUS GENERATION (*continued*).

The experiments of Jeffries Wyman: explanation of the results: Wyman's views on the subject. Dr. Bastian's views: Cases in which it is still possible that abiogenesis may occur: Growth in vacuo—Bastian's experiments—my own results—Cohn's facts—Dr. Roberts's objections, the walls of the vessels remain impure—Bastian's reply—Objections to the latter—Gruithuisen's experiments—Paul Bert's results with compressed air—Pouchet looks on a vacuum as preventing spontaneous generation—Paul Bert's results with rarefied air—Dr. Bastian does not always get positive results: Experiments in airless and hermetically sealed flasks raised to a high temperature—Objections—Prof. Huxley's and Dr. Sanderson's statements—Ray Lankester's results—Hartley: experiments with alkaline fluids—Roberts's counter-experiment. Mr. Lister's experiments. Experiments by Roberts and Tyndall.

OTHER writers, chiefly French and Italian, among whom may be mentioned Joly, Musset, and Mantegazza, have supported Pouchet, but as their experiments furnish little or no additional evidence nor new argument I think it unnecessary to discuss them. Those of Joly and Musset will be found in the 'Comptes Rendus de l'Académie des Sciences,' about the same period as the papers of Pasteur and Pouchet.

I must, however, refer at length to the experiments of Professor Jeffries Wyman, of Cambridge, U.S. These have been largely quoted by the supporters of heterogenesis, as proving their view, though it ought to be borne in mind that Wyman himself expressed no such opinion. It must be confessed that at first sight the experiments seem difficult of explanation on the Panspermic theory, and it is the more necessary to scrutinise them carefully, as he has evidently approached the subject with a perfectly unbiassed mind, and has therefore simply recorded his facts without attempting to force any definite conclusion on this question from them.

The following are the facts which have been adduced by supporters of spontaneous generation as favouring their views.

Flasks were prepared in three ways:

1. 'The materials of the infusion were put into a flask' (the general relation between the quantity of fluid and the capacity of the flask was, that about 20 c.cm of fluid were introduced into flasks of about 500 c.cm. of capacity) 'and a cork through which passed a glass tube drawn out to a neck was pushed deeply into the mouth of it. The space above the cork was filled with an adhesive cement composed of resin, wax, and varnish. The glass tube was bent at a right angle and inserted into an iron tube and cemented there with plaster of Paris. The iron tube was filled with wires, leaving only very narrow passage ways between them.'

Into these flasks such fluids as sugar, gelatine, and hay infusion—cheese, sugar, and gelatine—flesh, sugar, and gelatine, &c., were introduced, and boiled for periods varying from fifteen minutes to two hours, while at the same time the iron tube, filled with wires, was heated to redness. On withdrawing the lamp from the flask, the air which entered passed over these heated iron wires. When cold the flasks were sealed with the blow-pipe. Fourteen vessels were prepared in this way, and in ten of these, when opened after the lapse of various periods of time, organisms were found, generally vibriones and bacteria. The other four remained barren.

2. In a second set of experiments the cork in the neck of the flask was avoided, the neck itself being drawn out and bent at right angles, and into the orifice of this tube the iron tube was cemented. The other conditions were the same as in No. 1.

Similar fluids were used here as in the former case, such as gelatine and sugar with a few drops of urine and milk, beef infusion, &c. Thirteen flasks were treated in this way, and in all organisms appeared.

3. In others the flask was sealed at the ordinary temperature of the room, after the fluid to be tested had been introduced, and then it was submerged for a variable period in boiling water. This was a repetition of the experiments of Needham and Spallanzani. In all the flasks so treated organisms developed.

Four experiments were made under pressure, and of these two gave evidence of life ('monads and vibrios'), the other two remaining barren.

Such facts coming from an accurate and totally unprejudiced observer cannot be dismissed lightly. It is quite evident, on reading Wyman's paper, that the facts are accurately narrated, and we must therefore see whether any flaw can be detected in the method of experimentation, and we must attempt to find some explanation of results so diametrically opposed to those obtained by Pasteur which are, it must be remembered, equally indisputable.

Now, if we compare this method with that adopted by Pasteur, we shall see that with one exception the essential details are the same. This exception is, however, an extremely important one, and is probably the explanation of the diverse results obtained by several honest workers, and even by the same worker at different times. Pasteur takes a flask having a capacity of 250 to 300 c.cm., and into this 100 to 150 c.cm. of the liquid are introduced. Wyman uses flasks varying from 500 to 800 c.cm. in capacity, and into these he puts 12 to 40 c.cm. of the liquid. (In neither case was there any attempt at preliminary purification of the walls of the flask or of the air in the interior.) In Pasteur's experiments the fluid occupies $\frac{1}{2}$ or more of the capacity of the vessel; in Wyman's only $\frac{1}{20}$ to $\frac{1}{30}$ part.

Such is the only important difference between their methods; and this affords, I believe, sufficient explanation of the opposite results. For in Pasteur's flask only a proportionally small part of the wall of the flask has to be purified by the steam, and the extent of this part is of course much diminished by the ebullition of the fluid during boiling. There is also in Pasteur's flask only a very small quantity of air, with its dust, to be acted on. It is thus not to be wondered at that a barren result was obtained. But in Wyman's experiments by far the greater part of the flask and of its contents is impure, and can only be purified by the steam. Now steam, as heat, must be looked on as dry heat, and it is stated by Wyman, in a later publication,[1] that certain forms of organisms may

[1] *American Journal of Science*, vol. xliv. 1867.

resist the prolonged application of even a higher dry temperature than 212° F. Wyman also points out that the temperature of the air even half an inch above the surface of boiling water is many degrees below the boiling point. How much lower then will this temperature be at the orifice of this large flask during the greater part of the time in which the fluid is boiled? But even admitting that steam is moist heat (what I am by no means disposed to allow) several remarkable instances of vegetable growth at high temperatures are produced by Wyman, in one case even at a temperature of 208° F.

Such is the explanation I would give of Wyman's results, and that this is a true explanation will be very evident when I come to the consideration of the method of experimentation adopted by Mr. Lister. This explanation accords in every way with my own experience, in which I could point to several similar instances.

As I have said, Wyman is generally quoted in support of the theory of spontaneous generation, and at one time I thought that he had entertained that view, but the following facts brought to my notice by his brother, Dr. Morrill Wyman, show that he never gave any expression of opinion on this point, and that he appreciated the possibility of such an explanation of his results as I have given.

His first article is entitled 'Experiments on the formation of infusoria in boiled solutions of organic matter enclosed in hermetically sealed vessels and supplied with pure air,'[1] and his second, 'Observations and experiments on living organisms in heated water.'[2] With regard to the object of his research he says, 'The observations and experiments contained in this communication have not been brought together either for sustaining or refuting the doctrine (spontaneous generation) just referred to, but partly with the view of testing the accuracy of the experiments formerly made, and chiefly for the purpose of determining how far the life of certain kinds of low organisms is either sustained or destroyed in water which has been raised to a high temperature, a result which must be reached before spontaneous generation can be either asserted or denied.'

[1] *Silliman's Journal*, vol. xxxiv. 1862.
[2] *Ibid*, vol. xliv. 1867.

With regard to his experiments he says, 'In the first experiments the red hot tube, beyond a question, destroys all organisms contained in the air which enters the flask through it, but is without effect on such as may be contained in the solution, or adhere to the inner surface of the glass. These come in contact only with boiling water or steam, and unless destroyed by one or the other of these would be sufficient to vitiate any experiment, however careful the adjustment and heating of the tube may have been. We therefore believe that the tube is an unnecessary and useless complication of the apparatus.'

In another set of experiments it was shown 'That if the boiling of the flasks was continued for four hours, the infusoria may appear nevertheless—though in other cases it has happened that life ceased to be manifested if it was continued only for two hours.' 'In pushing the experiment still further, we have not found that infusoria appeared in any instance if the boiling was prolonged to five or six hours.' Several experiments, in which many flasks were used, were tried, but 'the result was uniformly the same. Thus a limit to the development of infusoria in boiling water was reached.' Dr. Wyman tells me that in the summer of 1880 he examined one of these flasks, which is marked as having been prepared in June, 1867, and which has remained unopened ever since. 'Judging by the signs above given' (absence of scum, of muddiness, or of fermentation) 'there is no evidence of infusorial life.'

The last defence of heterogenesis which it is necessary to consider is that by Dr. Bastian.[1] He gives up the theory of organic molecules *derived from previously living molecules*, and attempts to demonstrate that vital force and living matter may arise *de novo* under the action of the ordinary physical forces—heat, light, electricity, &c. This change of front on the part of the heterogenists is clearly brought about by the overwhelming evidence produced against Pouchet's views, and more especially by Pasteur's success in cultivating organisms from dust in fluids containing no organic matter. A further admission is made which somewhat simplifies the question, viz., that organisms have the power of self-multiplication.

[1] *The Beginnings of Life*, 1872, &c.

The limitation of cases of spontaneous generation, which has been gradually taking place, is exceedingly instructive. Beginning with the higher animals, it became gradually more limited, frogs, flies, &c., being by degrees excluded, till now it is only in the case of the lowest forms of life that the doctrine is asserted, and even there only in certain instances. The cases which are yet doubtful are given by Bastian in the work quoted, and may be grouped into three divisions.

I. The first division relates to the development of organisms in various fluids, more especially *in vacuo*—a condition which Pouchet looked on as inimical to life!

Into flasks portions of various infusions were introduced. The latter were then boiled for from ten to twenty minutes, and hermetically sealed while still boiling. The fluids used were turnip and hay infusions, and also solutions of certain salts, chiefly citrate of iron and ammonia containing portions of wood, cheese, &c.

The conditions of the first experiment mentioned are very striking and unusual.

'A closed flask containing a very strong infusion of hay (boiled for five minutes), *to which had been added $\frac{1}{20}$th part of carbolic acid*, was opened twelve days after it had been hermetically sealed.' Bastian states that this flask contained organisms of a peculiar form.

Such a statement as this, that a saturated solution of carbolic acid (for a watery fluid at the ordinary temperature containing $\frac{1}{20}$th part of carbolic acid is saturated) can *permit* the growth of organisms, is absolutely opposed to all experience and experiment. In experimenting with turnip infusion, cucumber infusion, &c., I have never been able to grow any sort of organism in these fluids, when they contained a larger proportion of carbolic acid than $\frac{1}{275}$th part, even though several drops of fluids swarming with bacteria were introduced. Further, I have lately performed the following experiment:—In January 1880 I introduced carbolic acid into flasks containing strong unboiled hay infusion so as to have a strength of the acid present, varying from 1 in 20 to 1 in 200 parts. These flasks were then covered with cotton-wool caps, and placed in an incubator. When examined six weeks later, there had not yet appeared in any one of them any sort of organism. And lastly, this statement, that organisms can develop in acid fluids after boiling, is contrary to the whole tenor of Dr. Bastian's later remarks, for his strong point is the development of organisms in alkaline—not in acid—fluids after prolonged boiling.

Bastian also employs turnip and hay infusions (without carbolic acid) and solutions of such salts as citrate of iron and ammonia, and he finds that a slight sediment occurs which contains organisms. He generally has to introduce such things as deal wood, cheese, &c., in order to get this result.

With regard to experiments on such fluids as hay infusion and turnip infusion without cheese, I may state that I have quite lately repeated them with exactly opposite results. At first I proceeded to repeat them, following closely Dr. Bastian's directions, in the expectation of getting organisms, and looking out for some explanation of their occurrence. The physical forces, or whatever else it may be, were, however, not favourably disposed for spontaneous generation at the time and place where I performed those experiments, for to my surprise I was unable to obtain any development of organisms. I tried several modifications, in the hope of finding the cause of their absence, but whichever of these vegetable fluids I used I was able, with proper precautions, to preserve them with the greatest ease. Some specimens were very difficult to filter, and in some a slight muddiness occurred on boiling, and the granular deposit might very readily be mistaken at first sight for organisms, though some care and experience would easily prevent such an error. But I have boiled the fluids for a few minutes and then filtered them under pressure (I could not in this way remove any of Dr. Bastian's supposed physical forces), and having thus obtained a perfectly clear liquid, I treated it like the others. There was now no deposit, and nothing which could be mistaken for organisms.

No doubt other observers have produced evidence which apparently at first sight supported Dr. Bastian's views. I refer to the class of experiments in which prolonged boiling was required for sterilisation, but many of these results depend, I believe, on the same causes as Wyman's, viz., imperfect purification of the walls of the flasks and of the air in their interior,

while the fact, that in some instances such resistance was met with, surely implies the presence of some form of encysted organism or resisting spore, or of an organism placed under conditions in which it is not perfectly heated, rather than some rare form of organic molecule or physical force.

The former view—that there is present in the infusions some form of resisting spore which can withstand the high temperature—was shown by Cohn to be correct in the case of the experiments where portions of cheese were introduced.[1] He repeated Bastian's cheese experiments with great care, and found that after exposure to a temperature of 100° C. for ten minutes, organisms still developed in the mixture of cheese and turnip. He, however, observed that these organisms were always of one form (*Bacillus subtilis*), and that *Bacterium termo* and other forms were absent. On investigating this subject further he found that these bacilli did not merely grow in the form of long rods, but that they produced spores, and he had previously ascertained[2] that the spores of these organisms were possessed of peculiar resisting powers. Indeed, such was their power of endurance under high temperatures, that if some satisfactory explanation could be given why they should always occur in these experiments, the whole mystery would be solved, and the theory of spontaneous generation would no longer be supported by these facts.

Cohn therefore turned his attention to the manufacture of cheese. The Swiss cheese is made in the following manner: milk is placed in large copper vats, and is coagulated by the addition of rennet. This is allowed to stand for a quarter of an hour, and then, after having been kept at a temperature of from 55° to 60° C. for an hour, it is broken up into small masses. These are now taken up in a cloth, placed in a mould, and pressed for twenty-four hours. The cheese is then taken out of the mould, transferred to a cellar, and kept at a temperature of 10° to 12° C. for several months, salt being daily rubbed over its surface. Lastly, it is stored till it attains its full ripeness.

[1] See *Untersuchungen über Bacterien, Cohn's Beiträge zur Biologie der Pflanzen*, Erster Band, Drittes Heft, p. 188.
[2] Cohn, *ibid.*, Heft 2, p. 176.

The only stage in the process which it is necessary to consider is the ripening of the cheese. Cohn points out that this is a true fermentation due to the growth of organisms; this

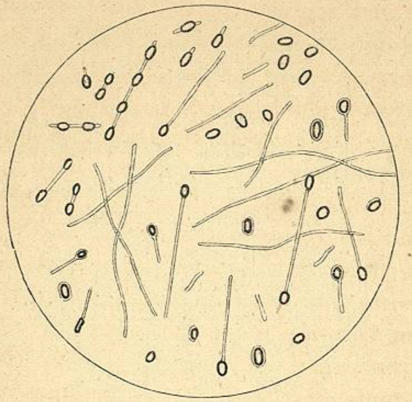

FIG. 64.—BACILLUS SUBTILIS; WITHOUT SPORES; WITH SPORES IN THE RODS; FREE SPORES; × 600 (AFTER COHN).

fermentation begins during the first twenty-four hours, while the curd is still under the press, and is accompanied by the development of large quantities of gas. The slower development of this gas, which occurs later, explains the formation of cavities in the cheese. The chemical change consists in the partial transformation of the milk sugar into butyric acid. The preliminary heating to 55° or 60° C. kills all the organisms except the bacilli which give rise to this butyric fermentation. On examining the rennet Cohn found that it was full of bacilli, many of which contained spores, and of free spores. By the addition of the rennet to the milk enormous numbers of these spores are sown in it, and the subsequent stirring mixes them thoroughly with it. These spores escape death at the temperature of 55°—60° C., and develop in the cheese, thus causing its ripening. They have been shown to resist high

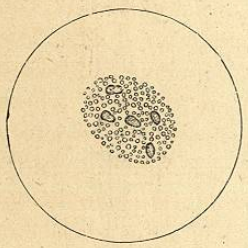

FIG. 65.—DEPOSIT IN RENNET, CONSISTING OF A MASS OF MICROCOCCI AND SPORES OF BACILLI, × 600 (AFTER COHN).

temperatures, and, when used in Bastian's infusions, they are not destroyed, and thus we have a satisfactory explanation of the frequent development of bacilli in these experiments.[1]

The other view—that the organisms were imperfectly heated—was urged against Dr. Bastian's experiments by Dr. Roberts as long ago as 1873. After pointing out that atmospheric germs may get into the flasks at the time of sealing, he goes on to a second source of error, which he considers much more important. 'It is this: Dr. Bastian's process does not insure that the entire contents of the flask are effectively exposed to the boiling heat.' He refers to the difficulty in boiling milk and other substances, owing to the spurting and frothing of the fluid; but he shows that if this is avoided by simply *immersing the flasks* in boiling water, the difficulty in rendering them barren is overcome. He says: 'The essential conditions of the experiment are first the effective exposure of the whole contents of the flask to a boiling heat; secondly, the absolute prevention of any fresh entrance of extraneous solid or liquid particles; and the conclusion I have come to is that if these conditions are rigidly observed, the flasks remain barren. If they do not remain barren it is simply because one or other of these conditions has not been observed.'

In answer to this Dr. Bastian[2] replies: 'I feel quite sure that in my experiments no portion of the inner surface of the glass has escaped the scathing action of the boiling fluid. The vessel has generally been more than three-fourths full before the process of heating has been commenced, so that where ebullition occurs the fluid has always swept over the previously uncovered inner surface and, as Dr. Sanderson testifies, "during the boiling some of the liquid was frequently ejected from the almost capillary orifice of the retort." The inner surface of the vessel was, in fact, always thoroughly and repeatedly washed with the boiling fluid, nearly half of which has been spurted away in order that I might effect this object.'

Now it is just the spurting of the liquid which is so danger-

[1] The experiments of Huizinga, on which Bastian lays great stress, have been refuted by Samuelson (*Pflüger's Archiv*, viii. p. 277) and by Gscheidlen (*Ibid.* ix. p. 163).

[2] *Nature*, February 27, 1873.

ous, for, with the bubbles, solid particles are carried up and deposited on the neck or sides of the flask, out of reach of the boiling liquid, and they may not be acted on by the frothing fluid. I also very much doubt if a bubble of steam sweeping over the wall is to be regarded as a very efficient way of applying moist heat; certainly it is not so efficient as boiling in a fluid. That greater success is obtained when this spurting and frothing do not take place has been stated by Dr. Roberts, and this statement is quite confirmed by Mr. Lister's experience with milk, where he uses the method of immersion with perfect success, and for the same reasons.

But surely this view, that the walls of the vessels remain impure, is the only way in which Bastian's facts can be reconciled with Gruithuisen's experiments mentioned by Bastian himself in a paper read before the Royal Society on March 20th, 1873. I will just quote Dr. Bastian's remarks and experiments in connection with this paper. It is to be observed that Bastian used this method for ascertaining the death-point of bacteria, and the title of the paper in which these statements occur is, '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.'

He says: '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 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 100 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.'

These bottles were kept at a temperature of from 65° to 75° F. The results were as follows:—

'The experimental results here 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° F. to a little below 158° F.), after an exposure to which the inoculated organic infusions are apt to become more slowly turbid, although