ART. XLVII.—*Experiments on the Production of Organisms in Closed Vessels*; by GEORGE CHILD, M.D.¹

THE researches, an account of which is contained in the following paper, are in continuation of those which, through the kindness of Prof. Phillips, I had the honor of communicating to the Royal Society in May last, and of which an abstract appeared in the 'Proceedings' for June 16, 1864. The former series of experiments did not pretend to be, in any respect, complete. Those which I am now about to describe will, I hope, be considered to be more so in regard to one main subject of the inquiry; but they also suggest further researches upon some collateral branches of it, which I hope to find time and opportunity to prosecute. In the former series I experimented with animal substances mixed with water and enclosed in glass bulbs

¹ From the Proceedings of the Royal Society for April 27, 1865, p. 178.

in atmospheres either of common air passed through red hot tubes or of various gases, and the result at which I arrived was that where oxygen was present organisms of a low type were produced, but not so where that gas was not present. Thus, whatever the gas employed, where the substance was not boiled, the organisms appeared; but in the instances in which the substance was boiled, they appeared where oxygen or common air was used, but not where nitrogen, hydrogen or carbonic acid was employed. One experiment only appeared to have produced a result which could not be reconciled with the rest, viz. in which some meat and water had been boiled and sealed up in an atmosphere of nitrogen. In this, some organisms were found; but so completely was this result unlike that found in the whole of the rest of the series, that I felt convinced that some error must have been made in the experiment itself.

The experiments now to be described have a narrower range than the others. With the exception of a few, which were mere repetitions of the experiments of nitrogen just referred to, and which were undertaken solely with the view of seeing whether the experiment just mentioned were correct or not, they are confined to the single object of observing whether or not organisms are found in close vessels containing vegetable matter and water sealed up in an atmosphere of common air previously passed through an efficient heating apparatus.

In these experiments I have adopted some slight modifications of the apparatus used in the former ones. That now employed consists of a porcelain tube, the central part of which is filled with roughly pounded porcelain; one end is connected with a gas-holder, and to the other the bulb is joined which contains the substance to be experimented upon. The bulb has two narrow necks or tubes, each of which is drawn out before the experiment begins, so as to be easily sealed by the lamp; one neck is connected with the porcelain tube, as already stated, by means of an india-rubber cork, and the other is bent down and inserted into a vessel containing sulphuric acid. The central part of the porcelain tube is heated by means of a furnace, and when it has attained a vivid red heat the bulb is joined on, the end of the porcelain tube which projects from the furnace being made thoroughly hot immediately before the cork is inserted, the cork itself being taken out of boiling water, and the neck of the bulb being also heated with a spirit-lamp immediately before it is inserted into the cork. A stream of air is now passed through the apparatus by means of the gas-holder, and bubbles through the sulphuric acid at the other end. The substance in the bulb is then boiled for ten or fifteen minutes, the lamp withdrawn, and the bulb allowed to cool while the stream of air is still passing through the porcelain tube, maintained

during the whole time at a vivid red heat. When the bulb is quite cool, the necks are sealed by means of a lamp. The advantage gained by means of this apparatus is that there is only one joint the perfection of which in any degree affects the success of the experiment, and that joint it is easy to make sure. The porcelain tube also being, for a considerable part of its length, filled with small fragments of porcelain, all heated up to redness, easily ensures that every particle of air admitted to the bulb shall be thoroughly heated. A precisely similar arrangement was used for the nitrogen experiments, substituting a glass combustion-tube filled with copper turnings for the porcelain tube, and a piece of india-rubber tubing for the india-rubber cork. The copper-oxyd. was reduced by means of a stream of hydrogen when necessary between one experiment and the next.

A single experiment was tried on May 18, 1864, using apparatus similar to that employed in the experiments of the previous year.

Some pea-meal infused in water was boiled in a stream of heated air, allowed to cool, and then sealed and put by. I was then prevented from resuming my experiments for several weeks.

Then several experiments were made with nitrogen, for the purpose of confirming or correcting the nitrogen experiment of the previous year. Into the particulars of these I need not now enter, further than to say that seven experiments were tried with various infusions. Five of them were afterwards examined, and in no case were any organisms found, thus confirming me in the opinion already expressed upon that experiment. The series with which I am now concerned began on July 18.

VII. July 18.—Hay infused in water three hours, then filtered and boiled 12 minutes in a stream of heated air, and sealed up as above described.

VIII. July 18.—A similar experiment: boiled 10½ minutes.

IX. July 22.—Toppings, *i. e.* coarse flour infused in cold water 8 hours, filtered and boiled 10 minutes in a similar stream of air.

X. July 22.—A similar experiment: boiled also 10 minutes.

XI. July 25.—A similar experiment: boiled 12 minutes.

XII. July 25.—A similar experiment: boiled 10 minutes.

XIII. July 28.—Some sage-leaves bruised and infused in lukewarm water previously boiled. Allowed to stand 15 hours, filtered, and the clear fluid boiled 10 minutes in a stream of heated air, as in the other cases, and sealed up.

XIV. July 28.—A similar experiment: boiled 7 minutes.

XV. July 29.—A similar infusion of celery, allowed to stand 12½ hours, and treated as the last: boiled 12 minutes.

The bulb used in this last experiment was of a different form, which I have found much more convenient, and have always

employed in my subsequent experiments, which are presently to be described. (It is represented in figure 1.)

The examination of the above series of experiments took place partly on Sept. 19, when Dr. Beale kindly visited me at Oxford, in order to give me his valuable assistance, and partly at Dr. Beale's home in London, on Nov. 16, 1864.

Exp. of May 18.—Viz. pea-meal and water. In this were found small organisms moving, as given by Dr. Beale in the accompanying drawing, figure 2. Their size was extremely minute, as they are here drawn as they appeared under a power of 1700.

Exp. VII.—Hay + water + heated air. Some large dumbbell-shaped crystals and a few bacteriums, very minute, but not so small as in the former case (fig. 3).

Exp. VIII.—The pair experiment to VII. Similar crystals, and organisms also similar, but larger.

Exp. IX.—Coarse flour + water + heated air. The result of this experiment was unsatisfactory, and serves well to show the difficulty of a decision upon these questions.

Even with the high powers above named, we were unable to be certain of our result in this and several following cases. There were no organisms distinctly recognizable as such, but many minute round spore-like bodies moving about the field.

Exp. X.—The fellow experiment to the last, and similarly unsatisfactory.

Exp. XIII.—Sage + water + heated air. A few crystals were seen, but no organisms.

Exp. XV.—Celery + water + heated air. Some prismatic crystals; no organisms.

It was resolved to leave the rest of these experiments till a longer time should have elapsed since the vessels were closed. The examination was accordingly resumed Nov. 16.

Exp. XII.—Coarse flour + water + heated air, contained some indeterminate granular matter, and some few bodies which might be dead bacteriums, but nothing that could safely be considered as such.

Exp. XI.—The fellow experiment to XII, and equally without result.

Exp. XIV.—Sage + water + heated air, gave also no definite result.

Now, omitting altogether the nitrogen experiments, seven in number, we have here a series of ten experiments instituted with a view of showing whether organisms can be produced in vegetable infusions within closed vessels supplied with heated air. In my desire to try a variety of substances I took almost

anything which my garden afforded, and in this way probably my selection of sage and celery may have been a bad one, as the aromatic ingredients of these plants may be supposed to influence the result of the experiment, especially as in a close vessel any volatile oil would be retained. If, therefore, the three experiments with these substances be eliminated, there remain seven experiments, one with pea-meal, two with hay, and four with coarse flour. Of these, five were examined on Sept. 19, and in three (viz. the pea-meal and the two hay experiments) the vessels were found to contain moving organisms. In two (those performed with coarse flour) none were found, and in the remaining two, examined on Nov. 16, also none were found.

In the meantime, when, from several of the above experiments having produced negative results, I looked upon the series as inconclusive, I instituted a fresh series of twelve experiments in the end of September, as follows.

The apparatus employed was the same as that used in the last series, except that I had some large double bulbs made for the present series. In other respects the process was the same as before.

Exp. 1. Sept. 30.—Hay infused $3\frac{1}{2}$ hours in water, filtered, and boiled 10 minutes in a stream of heated air—sealed up when cool.

Exp. II. Sept. 30.—Similar in all respects.

Exp. III. Oct. 1.—Similar.

Exp. IV. Oct. 1.—Similar.

Exp. V. Oct. 5.—Flour infused in warm water $3\frac{1}{2}$ hours and filtered: boiled 11 minutes, as before, and sealed.

Exp. VI. Oct. 5.—Similar: boiled 10 minutes.

Exp. VII. Oct. 5.—A similar infusion infused $6\frac{1}{2}$ hours, not filtered; boiled 10 minutes.

Exp. VIII. Oct. 5.—Similar.

Exp. IX. Oct. 7.—Flour infused $3\frac{1}{2}$ hours, not filtered: boiled 10 minutes in a stream of oxygen, and sealed as before.

Exp. X. Oct. 7.—Similar: boiled $10\frac{1}{2}$ minutes.

Exp. XI. Oct. 7.—Flour infused $4\frac{1}{2}$ hours and filtered: boiled 10 minutes in oxygen.

Exp. XII.—Similar.

On Oct. 8 this series of experiments was divided into two sets: [B], Nos. II, IV, VI, VIII, X, XII, were placed on a high shelf in my dining-room; the rest [A] in a hot closet, by the side of the cooking-stove, in the kitchen.

The object of the latter arrangement was to ensure the vessels being kept warm enough during the winter months; but the heat was, I have no doubt, too great. I saw the thermometer on more than one occasion over 140° Fahr., and have reason to believe that I did not see it at its highest. Moreover, the bulbs

here were almost wholly deprived of light. Thus, before opening the vessels, I had made up my mind that the results of the other half of the series were most to be depended upon. The temperature of the room in which they were probably never fell below 40° Fahr., and was generally between 50° and 60°.

The examination of the B division of this series took place at Dr. Beale's house, Feb. 7, 1865. The results were as follows :

Exp. IV.—Hay + water + heated air. A few bacteriums were found in active motion.

Exp. II.—Hay + water + heated air. Very large numbers of similar organisms were found.

Exp. VI.—Flour + water + heated air. Few were found as compared with the last, but still several in active motion.

Exp. XII.—Flour + water + oxygen. No organisms found.

Exp. VIII.—Flour + water + heated air (unfiltered). A good many bacteriums, similar to the others.

Exp. X.—Flour + water + oxygen (unfiltered). Some bacteriums, but not moving.

The other set of experiments was examined by me at Oxford on various evenings between Feb. 16 and March 8; but during some part of that time I possessed no object-glass of sufficient magnifying power to avoid all uncertainty in the results.

In both of them, viz. Nos. V and XI, I could find nothing like bacteriums. In the three others, viz. III, VII and IX, there were what appeared to me dead ones (but a dead bacterium is an object of which few persons who have seen many would think it very safe to be very positive), and in one only viz. No. I, an infusion of hay, were they numerous and moving. This I mention particularly, because the objects were very well seen, and moving actively in the first slide which I examined, and could be the better seen on account of the clearness of the fluid and the absence of granular matter; but upon examining several portions after the vessel had been open for a few minutes, though they continued to be seen in equally large numbers, all movement had ceased. They were examined with a $\frac{1}{2}$ inch object-glass of Messrs. Powell and Lealand. Now, if we omit from these two series of experiments those which I have already shown reason to distrust, we have, in all, seven in the first, and six in the second series, which seem fairly to test the question; and these having been examined by Dr. Beale, as well as myself, bacteriums were found and seen by both of us in three out of the first seven, and five out of the remaining six—in all, in eight.

Now, it may be asked, why the same or similar organisms were not found in the other cases, if the experiments were fairly tried? The answer is this, viz. that we do not know all the conditions under which they exist. It is pretty clear that they

appear more easily in some substances than in others. Thus, in the first series above described, it will be noticed that the four instances in which none were found were all those in which coarse flour was the substance used. In the remaining three, where pea-meal or hay were employed, there the bacteriums were seen. So also in the other series, the one case in which nothing was found, was a case in which flour was used, and in the remaining five the most numerous and distinct bacteriums were seen in the hay infusion. This may arise possibly from the fact that the infusion of flour is not so clear as the others, and always contains more granular matter; thus bacteriums are less easily distinguished in it: and, where doubtful, it is my practice to decide in the negative; that is to say, unless the bacteriums are clearly seen, I enumerate the experiment amongst those in which they are not found. Further, it is possible that in some infusions they may live and die sooner than in others, and in most of these experiments with flour there was a mass of indeterminate granular matter which might have contained the bodies of whole populations of bacteriums. Finally, it is quite possible that they might, if existing in small numbers, escape observation. Their minuteness is extreme, and observation of them far from easy. At any rate, positive evidence in a matter of this kind is of more value than negative; and the fact that in eight cases out of thirteen they have been seen, not by myself only, but also, by so accurate and practised a microscopist as Dr. Beale, is of more weight than our having been unable to discover them in the remaining five cases.

The question which now remains to be discussed is, how is it that the results above given so entirely disagree with those arrived at by Pasteur, and now, to a certain extent, vouched for by the Commission of the Academy of Sciences. I have observed all the precautions which Pasteur himself speaks of as "exaggerated," yet I have shown bacteriums to be produced exactly under the circumstances in which he asserts that they do not exist. I believe this discrepancy is very easily accounted for. Pasteur, in his memoir, speaks of examining his substances with a power of 850 diameters. Now my experience throughout has been that it is impossible to recognize these minute objects, with any degree of certainty, even with double that magnifying power. When once their existence on a slide is shown with a power of 1500 to 1700 diameters, it is quite possible afterwards to recognize the same object with a power of 750, but I have repeatedly failed to satisfy myself in the first instance with the latter power; and on the one occasion on which I enjoyed the use of an object-glass giving a power of 3000 diameters, I found the recognition of these very minute objects rendered very much more easy. On one occasion I tried

the effect of a power of 450 (not possessing one of 850), and found that all satisfactory investigation of such objects with such a power was impossible. Any person has only to examine the drawings which accompany this communication (in one particularly, that marked 2) in order to satisfy himself that to come to any conclusion as to the presence or absence of such objects as are there represented, with a magnifying power of little more than one-fifth linear measurement of that from which they are drawn, would be quite impossible. The Commission of the Academy of Sciences, which has not yet concluded its labors, has not, so far as its present report goes, concerned itself with the microscopy of the question; it has, in fact, confined itself to the dispute (which has almost become a personal one) between MM. Pasteur and Pouchet. It is worth noticing, that the fact so often referred to by writers on this subject, of the fluid in the closed vessels becoming cloudy or not as a test of the presence or absence of bacteriums, is not satisfactory; I have constantly predicted, from the cloudiness or clearness of an infusion, the presence or absence of bacteriums, and very frequently been mistaken—quite as often too in the former case as in the latter.

As to the conclusions which can be drawn from these experiments, I need say very few words. I can now have no doubt of the fact that "bacteriums" can be produced in hermetically-sealed vessels containing an infusion of organic matter, whether animal or vegetable, though supplied only with air passed through a red-hot tube with all necessary precautions for ensuring the thorough heating of every portion of it, and though the infusion itself be thoroughly boiled. But how far this fact affects the question of what is called "spontaneous generation" is quite another matter.

It seems clear that either (1) the germs of bacterium are capable of resisting the boiling temperature in a fluid, or (2) they are spontaneously generated, or (3) they are not "organisms" at all. I was myself somewhat inclined to the latter belief concerning them at one time; but some researches on which I am now engaged have gone far to convince me that they are really minute vegetable forms.

The choice therefore seems to remain between the other two conclusions. Upon these I will not venture a positive opinion, but remark only, that if it be true that "germs" can resist the boiling temperature in fluid, then both parties in the controversy are working upon a false principle, and neither Pouchet nor Pasteur is likely at present to solve the question of spontaneous generation. In truth, if Pasteur's facts are incorrect, the whole question is relegated to the domain of what the French Academy Commission calls "pure discussion;" and the one point which I claim to have established by their re-

searches is precisely that Mr. Pasteur's facts are inexact—not because his experiments were not most admirably performed, but simply because the magnifying power of his microscope was insufficient for the work to which he applied it. I desire to append two remarks to this paper. The first is, that the common *à priori* objection, which Mr. Pasteur so well expressed in his memoir, to heterogeny in all forms, viz. that it is a doctrine which has been gradually driven from all the higher forms of life in exact proportion as our observation of them has become more exact, until at last it has been compelled to take refuge in those lowest forms which we are almost or altogether unable to observe, is really of little or no force. Its cogency depends on analogy, and the analogy has no existence. It is quite equally to be expected *à priori* that if any forms of life are generated spontaneously, they will be the very lowest and simplest forms, and since these happen to be also the most minute, the objection loses its whole force. And it is also a thing to be expected that we should find only the lowest forms, the earliest, i. e. in the scale of existence, produced under the disadvantageous circumstances in which they must be placed in such experiments as those above detailed.

The other remark is this : that, so far as my present researches have led me, I cannot but look upon improvement in the construction of microscopes, and increase of their power, as the only way in which our means of investigation of such questions as the production of Bacterium is likely to be largely increased. The $\frac{1}{8}$ inch object-glass recently constructed by Messrs. Powell and Lealand, of which a notice has appeared in the Proceedings of the Royal Society, has already shown something like an appearance of structure in these minute objects, and leaves, I think, no doubt about their organic character.