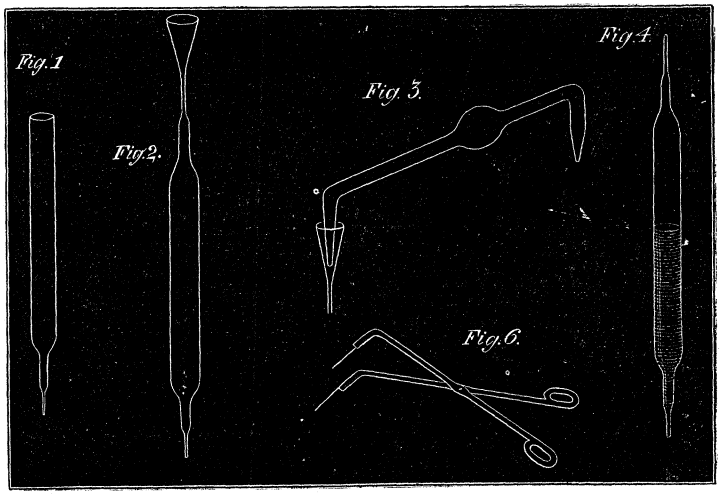
II. "Experiments concerning the Evolution of Life from Lifeless Matter." By WALTER NOEL HARTLEY, F.C.S., Demonstrator of Chemistry, King's College, London. Communicated by W. ODLING, M.B., F.R.S. Received December 7, 1871.

The work already accomplished, and the arguments adduced both in favour of and contradictory to the theory of spontaneous generation, have been so frequently under discussion of late, that it is needless to enter on a review of them. Furthermore, the question is one in which verbal argument is of little value compared with experimental evidence.

On June 30th, 1870, there appeared in 'Nature' a paper by Dr. Bastian, entitled "Facts and Reasonings concerning the heterogeneous evolution of Living Things;" the perusal of this, and its continuation, led to the belief that another interpretation might be put on the results obtained by Schwann, Pasteur, and others, not so much by virtue of the arguments made use of, as by accounts of experiments given in detail. The most remarkable case was that of Exp. 19, in which the author gave a drawing of a large organized mass obtained from a solution of sodium phosphate and ammonia tartrate, which had been exposed to a temperature varying between 146° C. and 153° C. for four hours. This organism was seen to grow within the flask till it attained a certain size, beyond which it did not increase. Now a fact so distinctly stated as the production of an organism, and its development to a considerable size, from a liquid containing nothing further than phosphate of soda and tartrate of ammonia, in a flask from which the air had been most thoroughly withdrawn, and which, when containing the liquid and hermetically sealed, had been heated to so high a temperature, was (admitting the conditions and performance of the experiments to be faultless) an absolute proof of the evolution of living matter *de novo*. For my own satisfaction, I determined to commence a series of careful experiments, in some cases adhering strictly to the conditions of those made by Dr. Bastian; but it was necessary to devise some refinement on the mode of examining the liquids experimented on without exposure to atmospheric air; the means for accomplishing this I will now describe. The most promising plan seemed to be, to open the sealed vessels in an atmosphere artificially prepared so as to be free of living matter. Hydrogen being fourteen times lighter than common air, may remain in contact with it without risk of contamination by floating matter; indeed Prof. Tyndall's demonstration, by means of a powerful beam of light, that such an atmosphere is free from dust, was sufficient to warrant its use. The means whereby this fact was made of further practical value are the following:—

1st. *The experimental tubes* in which the infusions and solutions were heated were made of ordinary combustion tubing drawn out at the lower end, first to a finer tube $\frac{1}{4}$ the diameter of the original, and after a space of an inch or so to extreme smallness. The solution or infusion was then made in a flask with distilled water, drawn by a siphon from a carboy

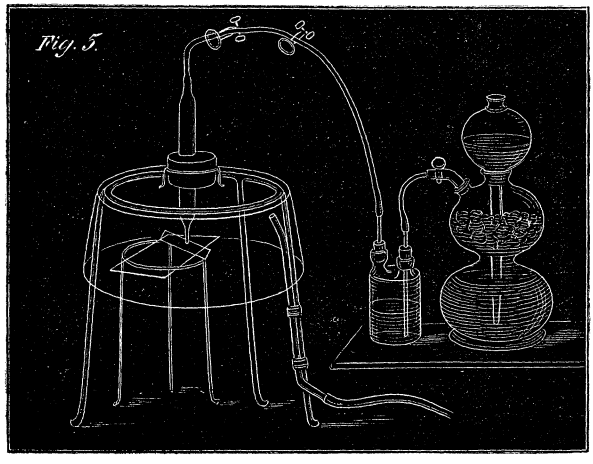
after standing at rest for many hours, the siphon dipping into the middle of the liquid. The flask was, after the usually careful cleansing that chemical vessels require, rinsed out with a solution of potassium dichromate mixed with strong sulphuric acid, then washed out with distilled water. A further quantity of distilled water collected in this vessel was used for the solution. The experimental tube, with the lower end drawn out but open, was cleansed with acid dichromate, and afterwards with hot distilled water. The fine point was open, in order to let the liquids run through, otherwise a drop might have collected in its capillary portion, and, not being dislodged, would have interfered with the experiment. This tube then, best described by fig. 1, had the fine end sealed up, and the liquid to be experimented on poured in till it about half-filled the tube. The upper part was then drawn out, so as to serve a purpose yet to be named, and also in such a manner that it could be adapted to the Sprengel exhauster; after this operation it had the form of fig. 2. It was then fitted to the Sprengel by means of a tube shown in fig. 3, which admitted of connexion by means of two Sprengel joints, the one over the experimental tube being made air-tight with water. The reason for water being used was this: had there been a leak, water would have entered the tube, and so no damage would have resulted; but had it been mercury or glycerine, the tube would necessarily have been rejected. The use of the bulb on the connecting-piece was to catch the water which boiled or distilled over during the exhausting process. After complete exhaustion, recognized by the clicking of the falling mercury, a blowpipe-flame was cautiously applied



to the fine tube till it fused thoroughly, and it was then drawn away from the other portion. What was so far accomplished was the sealing of a solution or infusion in a vacuous tube of the annexed form (fig. 4). The

tubes thus prepared were heated in an air-bath, consisting of horizontal iron pipes surrounded by two iron jackets. The tubes lying horizontally were not in contact with the liquid on the upper part; so, after being heated in the one direction for a period, at Prof. Tyndall's suggestion they were turned over and heated anew, so as to bring every part of the tube into contact with the heated liquid. These tubes had generally a bead of glass fused to one side, so that if the tube were heated with the bead uppermost, it had to be turned over and heated again, the bead being downwards. Generally four tubes were heated at a time; and one of these was soon after cooling opened to allow access of air, in order to observe whether any change occurred differing from any that might take place in the sealed tubes.

2nd. *Apparatus for examining the contents microscopically out of con-*



tact with air.—This consisted of a bell-jar closed at the top with a bung, and supported on a tripod; this bell-jar was kept filled with hydrogen by means of a self-regulating apparatus continually passing a gentle stream of gas into the upper part of the jar by means of a glass tube. The bung was bored with an eccentric hole; through this passed the tube containing the liquid for examination; the end of the tube passed very little below half-way down the bell-jar. Under this was a small tripod, on which rested a glass plate to be used as a stage for the microscope slips. The tube then being *in situ*, over the upper point was tightly slipped a piece of non-vulcanized india-rubber tube connected with a constant hydrogen generator. It was not deemed advisable to make use of coal-gas, because, had any lifeless organism been found in a tube, it might have been objected that a trace of benzole or naphthalene vapour or other impurity had been fatal to the experiment. On the india-rubber tube were two burette clips; now, by breaking the fine point within the india-rubber (a scratch with a

file being previously made upon it), hydrogen flows into the vacuous tube. Both clips are now closed, and by means of forceps, the hand being beneath the bell-jar, the lower end of the tube is broken off. No liquid, however, escapes, because the internal pressure is not much in excess of that of the atmosphere. The condition of things now can be explained only by the aid of a drawing (fig. 5) : the whole arrangement consists of a pipette containing the experimental liquid above and below, which is an atmosphere of hydrogen ; each drop of liquid expelled is received on a slip of glass in such an atmosphere. A drop of liquid is deposited by squeezing the space of india-rubber between the two clips, that clip nearest the bell-jar being open; before removing the pressure, it is again closed, and the partial vacuum made by compressing the india-rubber is filled up by allowing gas to flow in from the apparatus. This precaution prevents the possibility of the atmosphere of the bell-jar bubbling up into the tube after a little fluid has been discharged.

3rd. *Treatment of the glass slips.*—These are heated in an air-bath to about 200° C., taken out while hot with forceps, and placed on the stage in the hydrogen to cool, and kept there till wanted.

4th. *The glass covers.*—These are washed in the acid-dichromate solution, then in distilled water, and finally in alcohol, and, being picked up by a pair of peculiar forceps, are heated over an argand burner till very hot ; they are then held in the glass vessel full of gas till cool enough to use. The forceps I devised (fig. 6) have points of watch-spring steel, so that a thin glass cover may be firmly gripped without breakage. They are made by cutting a small pair of crucible tongs an inch or so before the part where they bend at right angles ; they have then two pieces of thin brass rivetted on, which are bent at right angles an inch or so from their ends ; the points are made by rivetting on pieces of watch-spring steel a little more than an inch in length. When these tongs are held in the position of scissors, that is to say with the thumb above the fore finger, the ends point downwards. To take up a glass cover, the forceps being in the position mentioned, the wrist is turned over from right to left while the elbow is raised, the glass resting on the lower point while the upper is closed down on it, may be safely held and conveyed to where it is wanted. After a little practice these pincers are easily used.

The advantage of the bung closing the neck of the bell-jar being bored eccentrically is, that by simply turning the bell-jar horizontally the pipette point delivering the liquid may be shifted from a glass slip on which a drop of liquid has been deposited to another clean one, or be made to turn through such an angle as to be out of the way of the glass stage, in order to transfer the solution to a flask for further experiment.

Tartrate of ammonia was prepared by neutralizing a tartaric-acid solution with aqueous ammonia ; this was mixed with phosphate-of-soda solution, made by dissolving carefully washed crystals of the salt in hot distilled water. The mixture containing about 5 per cent. of the two salts was

slightly acidified with tartaric acid. It was found that in no case should filtering be resorted to if possible, as the finest Swedish paper transfers myriads of its fibres to the liquid. Paper of nitro-cellulose (that is to say, Swedish filter-paper treated with a mixture of sulphuric and nitric acids) is disintegrated to a less extent than ordinary paper; and it is possible that, for special purposes, it might be of service in filtering a liquid so as to render it clear of fine particles, though no filtration will thoroughly clear a liquid. With proper care, however, filtration is unnecessary when dealing with solutions of definite salts.

On July 15th, 1870, a tube was half-filled with the above 5 per cent. solution of the mixed salts, and exhausted by the Sprengel. On trying whether it acted as a water-hammer on the following day the tube was split into fragments. The tubes require very careful handling, as the pointed ends give way readily.

July 16th, 1870.—Three other tubes were sealed up with the same liquid, the exhaustion in each case being perfect. The glass was heated to redness before introducing the liquid. After heating to 150° C. for four hours (during a few minutes the temperature accidentally rose to 180°), the tubes in a perpendicular position were placed at rest on a shelf, where the temperature was about 25° C.

Exp. I. The liquid, at first clear, after the lapse of three days had deposited flocculi. On October 7th one of the tubes was opened in the apparatus, and with the care already described. The gelatinous matter, on examination by a microscope magnifying 400 diameters, showed no signs of organization or structure of any kind. A small quantity was collected on a very small Swedish filter, and removed by means of a small platinum spatula. On heating it did not char, and was therefore inorganic matter; the usual blowpipe tests showed it to be silica. The disodic phosphate had attacked the glass; the silica deposited on standing, and hence the jelly-like mass. The remainder of the liquid was examined, but no trace of an organism could be detected. This tube had been kept exactly 84 days, during which time its temperature ranged between 18° and 25° C.

On July 21st, 1870, three other tubes containing freshly prepared ammonium tartrate and sodium phosphate were sealed up, the liquid being simply boiled to expel air; these were heated to 110° C. for three hours. Range of temperature 18° to 25° C.

Exp. II. No. 1 was opened October 7th, and examined with the same microscopic power. No living organism, and no trace of organic matter even, beyond a cotton-fibre and one or two small patches of indefinite and shapeless matter, which came from the solid residue of the liquid adhering to the glass within the capillary tube being charred in the act of sealing up. Several drops of liquid were examined at the lower point, the centre, and the surface, with the above results in the first case, but in the latter portions the liquid was absolutely clear. This tube had been kept 78 days.

Exp. III. No. 2, July 21st, 1870. Tartrate of ammonia and phosphate

of soda. The examination was made in the same manner. No trace of life was discernible. Time kept 78 days. Heated to 110° C.

Exp. IV. Tube containing phosphate of soda and tartrate of ammonia, perfectly exhausted. Sealed on August 6th, 1870. Heated to 140° C. for four hours. Opened October 8th.

Seven drops of liquid were taken from the tube, but no trace of living matter was discernible. The first drop contained a cotton-fibre and a small brown mass, identical in nature with that before mentioned. On October 12th a renewed examination was made with a higher power, $\frac{1}{8}$ inch, by Ross, kindly lent by Mr. Savory, which, with the eyepiece used before, gave an enlargement of about 600 diameters. A diligent search revealed nothing beyond what was before noticed. The contents of the tube were then allowed to run into a flask cleansed first with acid bichromate and then rinsed twice or thrice with hot distilled water, and finally dried in an air-bath. The flask and its contents were closed against dust of a coarse kind, which, though not floating in the air, might have fallen into the liquid. By inverting a small beaker over its mouth, it was allowed to remain with the unopened tubes at a temperature of 25° C. On November 3rd a small fungoid growth was noticed in the liquid, and on November 7th this had increased to $\frac{3}{4}$ inch in diameter. It proved to be a mass of *Pencillium* with abundance of mycelium-filaments interlaced. The fellow tube, sealed on August 6th and unopened, had nothing in it that the eye, aided by a lens and a powerful light, could detect. Several other tubes were prepared, but no further examination was made with the microscope. In no case, however (and some of the tubes had been sealed nearly six months), was any sign of life perceptible.

Unfortunately a very serious illness for some time prevented the continuance of my experiments. This work was done in the chemical laboratory of the Royal Institution. I cannot, therefore, omit giving my best thanks to Dr. Odling, and also to Prof. Tyndall, for their kindly interest and advice.

Part II.

Modification of Experiments.

In the renewed examination of liquids kept some time in sealed tubes, commenced in July 1871, a slight modification in the original method of proceeding was used. A bell-jar was chosen, the upper mouth of which was ground perfectly flat at the edge. Instead of inserting a bung with a hole in it to receive the tubes, a metal disk, with a wide metal tube placed eccentrically and projecting half an inch, was luted on to the mouth of the jar by means of grease, or, better still, what is known in pharmacy as *resinæ ceratum*. The glass sealed tube was then slipped into an india-rubber conical stopper, or rather ring *; for the thickness of it was so slight that

* These things are made and sold for the purpose of fixing the taps into beer-barrels.

a tube of any size could be made to fit it, either by the india-rubber stretching when the tube happened to be large, or by binding with a piece of copper wire when it fitted loosely. The pipette was scratched with a file at each end, and over the upper one was slipped a piece of india-rubber tube, attached to a tube of glass about $\frac{1}{4}$ inch in bore and 4 inches long, tightly packed with cotton-wool. The caoutchouc tube was pinched by a burette clip, and the extremity of the tube enclosed by the caoutchouc was broken at the file-mark. The vacuum was considered good if the india-rubber tube collapsed completely; the burette clip was opened, and filtered air thus admitted into the vacuous space. In order to render any thing that might be attached to the interior of the india-rubber tube harmless to the experiment, it was dipped in glycerine and the glycerine squeezed out of it, or treated in the same way with melted bees'-wax or paraffin. The pipette fits into its place in the disk by means of the flexible stopper. By closing the burette-clip, the tube can be broken at the lower point without more than a drop or two of the liquid escaping. After about one third of the liquid had been examined, one half of the remainder was allowed to run into a flask which had been previously heated to between 200° and 300° C.* The tube was then removed, and the fine capillary point, when possible, sealed at a gas-flame. The finer the point the more easily is this accomplished. A portion of liquid remains in the tube. On heating rather strongly a little of this is driven out, and then no air can pass to the remaining liquid without passing over red-hot glass, which readily melts together. The tube and flask were then placed side by side in a warm place to undergo further observation. If the tube, or class of tubes, were called A, after opening it was labelled A′, and the liquid out of it exposed to the unfiltered air, A″. The tubes and flasks labelled thus were kept in a cupboard, the bottom of which was the metal lid of a long water-bath. It was thought better not to place the flasks or tubes in water, because the aqueous vapour which would thus surround the mouths of the flasks would create an abnormal atmosphere which might or might not affect the experiments; besides, such a plan is not so cleanly. The objective made use of was obtained from Messrs. R. and J. Beck. It was a $\frac{1}{8}$ glass, without any immersion-arrangement, and gave, with the second eyepiece of one of their microscopes, a magnifying-power of 750 diameters. Occasionally, for convenience in drawing, a power of 420 diameters was employed.

Method of examining a liquid which it was difficult to retain in the pipette-tube.

When it happened that the finely drawn-out end of the pipette was too large to retain the liquid, it was allowed to run into a small glass vessel, really a beaker cut down so as to measure about $1\frac{1}{2}$ inch in diameter and 1 inch high. Drops of the solution were removed from this to the glass slides while it stood on the glass stage in the jar of hydrogen, by means of

* That is to say, baked in an oven the bottom of which was red-hot.

a tube like a very long-legged siphon, at the lower end of which was a piece of caoutchouc tube, with one end stopped by a little piece of glass rod. This was nothing more than a bent pipette; by compressing the india-rubber when the pointed tip of the shorter limb was dipped into the beaker-glass, and then releasing it, the liquid entered for the space of an inch or so, and could then be easily transferred to a glass slip. It was thought as well to blow hydrogen through the tube before use; and, of course, like all the other apparatus, it was carefully washed and heated.

Preparation of solutions.

The water used was very pure distilled water taken from a carboy, the contents of which had been tested with a beam of light, and found to reflect chiefly the blue rays. A previous attempt to obtain pure water by distillation with sulphuric acid and potassium permanganate, in glass vessels and an atmosphere of hydrogen, did not yield better specimens. It is impossible to prepare solutions of salts which do not show abundance of floating matter to a ray of light, even when such pure water is made use of. Solutions filtered through the finest Swedish paper are crowded with fibres, which may readily be seen by filling a globular flask with the solution, the eye and an argand burner being on the same horizontal line, and about a foot apart. The flask is interposed, and gradually lowered till the particles are seen brilliantly illuminated on a dark ground. The phosphate of soda used was recrystallized immediately before being dissolved, and the tartrate of ammonia was prepared from recrystallized tartaric acid and the strongest aqueous ammonia. When the solutions were mixed, the alkaline reaction was neutralized by tartaric acid, or rendered faintly acid. Of course it is of the first importance that the tubes, after being sealed, should be heated immediately to the temperature necessary to destroy life, and this was done in every case as soon as possible.

Accidental occurrence of a lifeless organism in a phosphate-of-sodium and ammonic-tartrate solution.

On examining the contents of tube A 2, several drops of liquid were found to contain nothing whatever. They had filtered through the gelatinous silica deposited in the capillary point, so that a fresh portion of the glass had to be broken off and a little of the liquid allowed to run into a bottle. After this a little débris was occasionally noticed, and in one drop of liquid an animalcule was found, measuring 0·003 inch in length. I failed to recognize it, probably from its being injured by the action of a liquid at so high a temperature as 150° C. Its presence in the solution is more easily accounted for than if it had been found in any of those prepared subsequently; for in this case the water was not tested by means of a beam of light, neither was the glass tube (although well washed with boiling water) cleansed with such very great care. There was much less débris of the nature of cotton-fibres and other indefinite matter found in the tubes

prepared subsequently. It is at any rate satisfactory to know that it was lifeless, and the only thing of the kind met with. It is impossible to get glass apparatus quite clean by means of hot water; those in the habit of making organic analyses are acquainted with the large amount of dirt which can be swept out of a tube by a plug of filter-paper or cotton-wool, after washing with abundance of boiling water.

Experimental Results.

The whole of the tubes, an account of which here follows, were kept for sixteen weeks at a temperature of 30° *to* 34° C. *during the daytime, and not lower than* 20° C. *at night. A fluctuating temperature is considered by Dr. Bastian to be favourable to evolutional changes*. From the month of February till the time of examination the temperature would never be lower than* 16° C., *and was never higher than* 30° C.

A. A solution containing about 4 per cent. of a mixture of sodium diphosphate and ammonic tartrate, and having a neutral reaction, was placed in three tubes, which were exhausted with a Sprengel, sealed up, and heated for three hours to 150° C. Prepared on July 16th, 1870.

No. 1. No trace of any organized matter discovered; nothing but a little silica dissolved out of the glass. Opened October 18th, 1870. Time kept three months.

No. 2. Examined July 17th, 1871. Kept over a year. No living organism found. In this tube the animalcule already mentioned was met with. The liquid was turbid with silica.

No. 3. Examined September 2nd, 1871. Kept a year and six weeks. Nothing found. The silica makes these tubes troublesome to examine, on account of the capillary point tending to become stopped.

B. Tube prepared July 15th, 1870. Solution of salts, as in preceding experiments. Air expelled by boiling for ten minutes. Heated to 130° C. Kept one year and two months. Examined September 12th, 1871. Vacuum good. Nothing noticeable seen.

C. Same solution, but with decidedly acid reaction. Prepared August 8th, 1870. Opened July 17th, 1871. Kept ten months and twenty-five days. No trace of any organism; many drops of liquid absolutely free from any thing whatever. The lower end of this tube was a little too large, so that, in spite of the upper end being closed, the liquid would drop out. It was caught in a little glass vessel placed in the bell-jar, and drops were removed as required by dipping the point of the tube in and touching the glass slide.

D. October 10th, 1870. A strong infusion was made by pouring warm distilled water over finely shred turnips, and allowing to digest for some time. The liquid was filtered twice through the finest Swedish paper, and

* See 'Nature,' vol. ii. p. 177.

allowed to stand for some hours, when a portion was siphoned off, placed in tubes completely exhausted with a Sprengel pump, and sealed up. The tubes were heated to 120° C. for three hours. The tube marked T, and called the "test-tube" in these and all the following experiments, was treated in just the same way as the others, and heated at the same time to ascertain whether a high temperature had any prejudicial effect on the development of life.

No. 1. Opened July 14th, 1871. Kept nine months and one week. Vacuum perfect. Liquid perfectly clear; flavour and smell like that of fresh infusion. No change had taken place.

No. 2. Opened July 15th, 1871. Kept nine months and eight days. Vacuum perfect. Liquid quite fresh in smell and flavour, and clear in appearance. Quite unchanged.

No. 3. Opened August 4th, 1871. Kept over ten months and two weeks. Vacuum perfect. Liquid perfectly fresh in smell and flavour, clear in appearance. Quite unchanged.

No. 4, T. Opened October 12th. On 19th, in a space of seven days, the liquid had become turbid. On October 31st it was crowded with white matter, and had a very offensive smell. The microscope showed masses of *Torula*-cells. The magnifying-power used was 400 diameters.

The original solution, which had been kept covered by an inverted beaker over the mouth of the flask, was turbid six days after its preparation. As turbidity was noticed in the solution, which had been sealed *in vacuo* and heated to 120° C., in less than seven days (the tube being opened at night and examined in the morning) after exposure to the air, we see that heating had no interference with the experiment.

E. October 12th, 1870. Sodium phosphate and ammonic tartrate. Solution containing 3 to 4 per cent. of the mixed salts. Completely exhausted with a Sprengel pump. Heated three hours to 130° C.

No. 1. Examined August 24th, 1871. Kept ten months and two weeks. Vacuum perfect. Many drops of liquid free from any thing. Quite unaltered. Contained nothing noticeable.

No. 2. Air expelled by boiling. Vacuum good. Opened July 19th, 1871. Kept nine months. Remarkably little solid matter seen: no organism met with.

No. 3, T. Opened October 18th, 1870. Exposed to the air twenty days. Not examined till November 7th. Five masses the size of peas were seen floating on the liquid; they proved to consist of mucor, with fructification.

F. October 18th, 1870. Urine boiled and filtered from mucus. Each tube exhausted with the Sprengel. Heated to 130° C. for three hours. After heating, what is believed to be a trace of phosphate of lime separated.

No. 1. In February 1871 the liquid was perfectly clear and seemingly unaltered; when next examined the point of the tube was found to have been broken and the liquid had been lost. Kept about fourteen weeks.

No. 2. Opened August 29th, 1871. Kept over ten months and a half. Nothing unusual was seen in the liquid; it smelt perfectly fresh, and looked quite bright and clear.

No. 3. Opened July 19th, 1871. Kept nearly nine months. The liquid was quite fresh and clear. There were seen two or three minute bodies, too small for any definite observation to be made concerning their form. They had apparently an irregular rotatory slow motion, which continued; and careful observation led me to conclude it was only the Brownian movement. This conclusion was afterwards confirmed. See F′ 3.

No. 4, T. Opened October 19th, 1870. Both *Torula* and mucors were discovered in abundance after three weeks' exposure to the air.

Liquids which, after prolonged preservation in sealed tubes, were exposed to air filtered through cotton-wool, kept at a temperature of 30°–34° C. *during the daytime, and not below* 24° C. *at night.*

D′. No. 1. Examined on July 21st, and again September 7th, 1871.—The liquid was quite unchanged. Nothing found in it after a period of fifty-two days.

No. 2. Examined August 22nd and September 8th. No trace of any organism. Quite unaltered. Kept fifty-four days.

No. 3. As the first sign of any change occurring in the solution is turbidity, this tube was not submitted to microscopical examination. On September 8th it was still perfectly clear to the eye, apparently quite unaltered after more than a month.

E′. No. 2. Opened July 19th, examined September 13th, 1871, after a period of two months. Quite unaltered.

F′. Examined after exposure to air for five weeks, from July 19th to August 23rd, 1871.

No. 3. The liquid smelt quite fresh, and was unaltered in appearance. Only two of the minute particles previously mentioned were seen: they were most certainly lifeless, and also for the most part motionless; one was seen to move with a current in the liquid, but the rotatory motions before noticed did not occur. A most attentive examination for a length of time was devoted to these bodies; in size they could not have been larger than 0·00002 inch. The conclusion as to their being lifeless was borne out by the fact that their small number (only five were seen) had not increased, though under favourable circumstances for reproduction, even after a period of five weeks.

No. 2. Sept. 13. Liquid quite unaltered. Kept nine weeks.

Liquids which, after prolonged keeping without development of life, were afterwards exposed to ordinary air at a temperature ranging between 24° C. *and* 34° C. *Any pipette or glass rod placed in these liquids had immediately before been heated to at least* 200° C.

D″. Examined July 18th, after exposure to the air four days. No. 1. The liquid was very turbid, and smelt very offensive; it swarmed with

vibriones and some excessively small bodies, all in rapid motion. An immense number of minute bodies, 0·000025 in length, consisting of two cells, which are believed to be *Torula* in an early stage, besides one or two full-sized *Torula*-cells, were noticed. On July 21st an immense quantity of *Torula* and some fibrous growth, most probably fungus or conferva, appeared in addition to the other minute organisms. The liquid was boiled violently for a few minutes, but not longer, as the liquid frothed much. The vibriones remained as lively as ever.

No. 2. Examined August 1st. Nothing observed; liquid unchanged. August 22nd the liquid had evaporated to some extent. A thick coating of *Mucor** and *Torula* covered the syrupy solution. Kept seventeen days without alteration; total time exposed to air more than five weeks.

No. 3. Opened August 24th. On August 27th the liquid was observed to have a very slight sediment; later on a downy growth was observed adhering to the side of the flask. On the following day, August 28th, the liquid, which had become very turbid, was examined. It contained immense quantities of the undeveloped *Torula*. None of these bodies were seen to move. Reexamined September 1st; the tuft of down proved to be conferva. On September 4th the liquid was a mass of *Torula*. The confervoid growth had increased very little; there was a quantity of protoplasmic, perhaps germinal, matter distributed through the liquid, which was invisible in many parts until stained with carmine.

E″. No. 1. On August 28th, after three days' exposure to air, some specks of white matter were noticed adhering to the sides of the little beaker-glass in which the liquid was placed. On removing and examining them with the microscope, they seemed to consist of spores of fungi germinating.

No. 2. Examined July 29th, 1871. Exposed to the air ten days. A small mass, like a tuft of down, was observed with the unaided eye; it proved to be a collection of mycelium-filaments, and *Mucor* with fructification. Reexamined August 22nd. A mass of green conferva covered the liquid, and both *Torula* and *Mucor* were present.

F″. No. 2. This liquid was examined on September 2nd, 1871, after barely four days' exposure to the air. There were many bodies such as I have described as undeveloped *Torula* (Exp. D″, No. 1). On September 4th there were many sarcinæ to be seen, besides vibriones and other very minute bodies, in the greatest state of activity. Some of them seemed to attach themselves by one end to a point, and swing themselves round and round at a great speed; they did not measure more than 0·00005 inch in length. There were seen some "figure-of-eight" particles in rapid motion; they differed from the minute *Torulæ* by being less oval in form, and capable of moving rapidly.

No. 3. The quantity of liquid was very small. On August 23rd it had become very turbid; instead of examining it with the microscope, an addi-

* Well represented by fig. 12, *a*, p. 197, 'Nature,' vol. ii.

tional quantity was added from tube F″ 3 ; and on August 25th it had become filled with white matter, consisting of chain-like bodies, most probably motionless *Spirilla*.

It may doubtless appear to some that my particular mode of experimenting involves the introduction of needless complications, that others have obtained the same results without the use of such apparatus ; but the incorrectness of this will be admitted when I say that I was not wholly prepared for such a result, and the necessity of being guarded, of being safe, indeed, from every possibility of error, is absolute. Had living things been discovered, that the experimental method and apparatus was a matter of the first importance would have been evident, inasmuch as it excluded all possibility of atmospheric contamination of the experimental liquids during examination. In a word, it was necessary to be prepared for any result, and be guarded on every side. The foregoing description records all the experiments I have made with the view of obtaining the information I sought. It will be noticed that the evidence afforded is perfectly concordant.

The appearances described in Exp. 20*, by Dr. Bastian, are, with the exception of the fungus-spores and bicellular bodies, exactly what one sees in silica. Having ascertained the fact that phosphate of soda, and especially when not neutralized with acid, attacks glass tubes at a temperature of 150° C., and as in this case sodic phosphate and ammonic carbonate were heated for four hours at 146° C. to 153° C., I examined the silica deposited from my own tubes, and observed the gelatinous matter resembling such to be met with in solutions containing infusoria : there were two or three transparent spherules also which I believe to be water enclosed in silica ; they are often seen in pectized silica. As for the matter becoming stained by magenta, that is no evidence of its nature, this property being shared by silica†. It may further be remarked that magenta would be precipitated on addition to such a solution by the alkaline phosphate and carbonate : those parts more deeply stained than the others would be those where rosaniline was precipitated ; it would be impossible to use a salt of rosaniline for the purpose successfully. The nature of the solution, too, is such that no life will appear in it, even on exposure to the air. An alkaline solution of phosphate of soda and an ammonia salt was kept ten months open to the air, yet no change took place, while several other portions were kept for a shorter period at a temperature of 24° to 34° C. with a like result.

Dr. Bastian considers he has established by experiment the theory that living organisms, amongst which are vibriones and fungi of the genera *Mucor*, *Penicillium*, and *Torula*, and algæ, such as conferva, are evolved *de novo* from lifeless matter ; he brings together a number of reasons, of a more or less decided kind, to show not only why it should be an intelligible

* 'Nature,' vol. ii. p. 200.

† Journal of the Chem. Soc. vol. ix. p. 452.

process, but also why others, and particularly M. Pasteur, have obtained results leading to directly an opposite conclusion. These arguments are not drawn from experimental evidence; they do not therefore fall within the bounds of this discussion; but I would point out that, in one case, not only do Dr. Bastian's own experiments deny the truth of a most important assumption of the evolutionists, but also at the same time my own experience proves the contrary. He says*:—"The disruptive agency of heat is fairly enough supposed by the evolutionists to destroy some of the more mobile combinations in each solution—to break up more or less completely, in fact, those very complex organic products whose molecular instability is looked upon as one of the conditions essential to the evolutional changes which are supposed to take place." Before granting such a supposition, it would be necessary to know, first, what are "the very complex organic products" of such peculiar "molecular instability" existing in a solution of tartrate of ammonia, sodic phosphate, acetate of ammonia, oxalate of ammonia, in a solution of sugar and calcined yeast, in turnip infusion, or any other putrescible liquid. My experiments show that there is no such disruptive agency in a high temperature, that it does not influence the "more mobile combinations," either in solutions of organic salts or vegetable infusions; for the "test-tubes" T were precisely the same as the others of the series, contents identical, heated at the same time to the same temperature, in fact taken from among them indiscriminately, the only difference being that one was exposed to the air and the others were not. Besides, there is in addition the evidence afforded by the tubes classed under D″, E″, and F″; yet we find the changes occurred in them as readily as in the unheated original solutions. Dr. Bastian records† the development of organisms in a liquid heated as high as 153° C.; yet the assumed "disruptive agency of heat" is supposed to have influenced the results of Schwann and Pasteur at a temperature of 100° C.! His experience is contradictory to his own theory, and at the same time to the experiments of others, to which his theory raises objection.

It has long been established by Pasteur, Payen, and other experimenters, that a temperature of less than 130° C. is insufficient to destroy all trace of life if the germs or spores are not immersed in a liquid; this is a fact admitted on all sides‡. In that case it is not difficult to understand how, in the experiments of Dr. Child and Prof. Wyman, organisms have been found in liquids to which air only which had passed through red-hot pipes was admitted. The former took a bulb with two narrow necks or tubes, and containing the experimental liquid; one tube was connected by a cork boiled in water with a red-hot porcelain tube filled with pumice, and connected with a gas-holder; the other tube dipped into sulphuric acid: the

* 'Nature,' vol. i. p. 176.

† 'Nature,' vol. ii. p. 200.

‡ 'Nature,' vol. ii. p. 170; Amer. Journ. Science, vol. xxxiv. p. 79; Proc. Roy. Soc. vol. xiii. p. 313.

liquid was boiled for ten or fifteen minutes, and heated air was made to pass through the apparatus till the liquid and flask were cool. "When the bulb is quite cool, the necks are sealed by means of a lamp." In Wyman's experiments an apparatus of much the same kind was used; but the liquids in four cases were boiled for from five to ten minutes in a Papin's digester under a pressure of from two to five atmospheres, or at a temperature of 120° to 150° C. Large flasks of 500 cub. centims., and even 850 cub. centims. capacity were used, containing 17 cub. centims. to 50 cub. centims, or thereabouts, of solution, so that in some cases only $\frac{1}{25}$ of their capacity was occupied; the air admitted was passed through red-hot iron pipes filled with iron wires. There is a similarity between these two sets of experiments; the flasks were not entirely in contact with the hot liquid. We see also, from the few words quoted from the description of Child's experiments, that a sufficient space intervened between the red-hot tube and the bulb to allow of the heated air becoming cool before it entered the glass bulb; it cannot be said, therefore, that the entering air was so hot as to destroy whatever living thing might be attached to the glass. The precautions taken, then, were not sufficient to render the experiments trustworthy, more particularly in the case of Wyman's work, because there he had an immensely large surface untouched by fluid, and naturally he obtained more results in favour of the view of evolution than any other experimenter. Bastian's own experiments are open to the same objection*; in fact it seems that much work has been rendered faulty by this neglect of bringing every part of the interior surface of the containing vessels in contact with the heated liquid. We have positive proof that such is the case when, as Bastian himself states, "it has long been known that a boiled fluid extremely prone to change will not yield infusoria if the vessel in which it is contained is filled with the fluid;" the commercial method of preparing cooked meat depends upon this to a great extent. With respect to the power of vibriones to resist the destructive action of heat, I at one time felt sure, from experiments on hay infusion and decomposing turnip infusion, that they were capable of living after being boiled. This hasty conviction at the moment of observation arose from the fact that the movement of these bodies was the same before and after boiling, and at the same time unlike any example of the Brownian movement with which I was familiar; but there is no evidence that they were really living in the first case; it is only a presumption.

A strong infusion of hay was made with lukewarm water, filtered twice through Swedish paper, and examined under the microscope; it swarmed with life, especially in the form of bacteria, mostly in rapid motion; some of these were excessively small, less than 0·00005 inch in length. The liquid was boiled violently for fifteen minutes until two thirds had been evaporated away; a drop placed under the microscope showed that most of

* It is worthy of remark that in two out of the four cases in which Wyman heated liquids at a temperature over 100° C., no organisms were found.

the living bacteria had been killed, or were, at any rate, motionless. Careful observation, however, showed the minute vibriones to be as lively as ever; they curled and twisted, and dived out of focus just as when alive. The same fact was noticed in a turnip infusion. Pasteur proved that the germs of vibriones in milk were not killed till the liquid was heated to 110° C.* I fancy that the recent experiments of Dr. Crace-Calvert† lead him to fix the fatal temperature at too high a rate (between 150° and 200° C.), supposing the vibriones to be immersed in the liquid; but if any of them be left adhering to the glass tube, possibly not. The fact that hay infusion contains, besides such multitudes of living things, great quantities of organic débris, led to the conclusion that no safe experiments could be made with such a liquid. If a liquid be boiled in a flask and sealed up, it depends very much upon the nature of the liquid whether life will be developed in it: thus, if we take a most carefully prepared saline or sugar solution, the water used being particularly pure, and the salts dissolved and recrystallized with all possible care, the chances are that nothing will come of it; but if, on the other hand, a liquid swarming with life be chosen, the conditions will be most favourable for depositing living things on the sides of the vessel, where they will be out of reach of the boiling liquid, and, getting washed off into the liquid when cool, will there multiply. On this account, the use of hay infusion in particular has led to erroneous deductions, from faulty experiments. To any one who will make a strong infusion of hay with lukewarm water, and examine it with the microscope, it will, I am sure, be simply inconceivable how any appearance of accurate evidence can be derived from such a fluid, particularly when the liquid is not heated above the boiling-point.

It must be allowed that Pasteur's experiments prove that when fermentable liquids were protected from matter floating in the air, fermentation would not take place. Now the first evidence we have of fermentation in, for instance, a saccharine solution, is the presence of *Torula*-cells; moreover, to set up fermentation, the *Torula* is placed in saccharine fluid, the operation of every brewery. It is evident, then, that either the *Torula* is the agent of fermentative change, or is closely connected with it. But whence arises the *Torula*? From matter floating in the atmosphere? in that case, seeing they are invariable and definite organisms, which are produced from variable liquids, it must be from invariable and definite matter that these organisms arise. Whence come, what is the origin, the nature, the chemical composition, or, more particularly, the chemical constitution of the so-called dead organic particles of this invariable nature, producing such invariable yet tremendous changes in such very simple substances, and to what class of chemical compounds do they belong? Until this question has been satisfactorily answered, ordinary reason would assign to them the possession of life, for their properties are the properties possessed by none but living things. If these organic nitrogenous particles are not living,

* Ann. Chim. Phys. tom. lxiv. p. 61. † Proc. Royal Soc. vol. xix. p. 472.

how are we to account for the action of various antiseptics? In the Report to the Cattle-Plague Commissioners "On Disinfection and Disinfectants," by Dr. Angus Smith, an account is given (p. 10) of the action of a number of essential oils, such as oil of bitter almonds, oil of mustard, amylic alcohol, cresylic and carbolic acids, and ether, the vapour of which was diffused in air surrounding pieces of meat. Many substances had the property of preserving the meat for a great length of time, especially amylic alcohol and oil-of-bitter-almonds chloroform; and carbonic tetrachloride also shares this property. If these nitrogenous particles are not living things, how are we to account for the action of the substances? It cannot be a chemical action, because these substances are chemically inactive. In some cases the less active colytic agents having diffused away out of the bottle, mould formed on the meat, or putrefaction commenced; and this always happened on that part nearest the cork, showing that particles causing the change came in with air after the preservative agent had escaped. That these colytic agents were fatal to the growth of mould, or the spread of putrefaction, was ascertained by placing mouldy paste under a bell, the atmosphere of which contained a small quantity of the vapour.

It has been doubted lately whether carbolic acid has the power of destroying germs, whether, in fact, Prof. Lister's surgical treatment depends upon this power. The experiments which have given rise to the question tell us only that when carbolic acid is added to a putrescible liquid it is not preserved. There is nothing new in this. "The phenylic and cresylic alcohols, which experience has shown to be generally so active in the prevention of putrefaction, are not sufficiently constant when a large quantity of water is present"*. When in a state of vapour very greatly diluted with air, we find them preventing organic growths and putrefaction in an extraordinary manner. In order to preserve liquids, corrosive sublimate, sulphate of copper, and chloride of zinc are the most efficacious substances.

If those particles which are the origin of life are themselves lifeless, they could not influence the nature of the organisms developed; in that case different solutions would give rise to different organisms, and, conversely, the same solution would yield the same form of life. But we do not find this to be the case; for in the same infusion of turnip in Experiment D″ 1 and D″ 3 occur on exposure to the air at different times different forms of life. In the one case minute vibriones and masses of *Torula*-cells occurred, whereas in the other confervoid growth and some minute motionless organisms which had not previously been observed were the most noticeable forms. With different portions of a solution of alkaline phosphates and tartrates, in one case we got a fungoid and in the other an algoid form of life. Were it not for the great preponderance of sound experimental evidence to the contrary, it would be easier to believe the theory that life was evolved *de novo*. It is the crude idea which a superficial observer of everyday phenomena would entertain; the popular error that maggots are bred

* "On Disinfection and Disinfectants," Cattle-Plague Report, pp. 14 & 15.

out of corruption illustrates this. The theory involves the discovery of a new property of matter, the property that certain compounds (undefined nitrogenous particles in the atmosphere) must have of decomposing molecules of other substances with which they are in contact, and building out of their constituent atoms substances of a much more complicated nature, without the exertion of external forces; even beyond this, they must be capable of arranging those compounds into definite forms. In the course of nature complex substances do not increase but simplify their molecular complexity. By oxidation vegetable products are resolved into carbonic acid and water: we find no tendency in these oxides of carbon and hydrogen to become reduced to such products as sugar, starch, or cellulose; nay, much further, we know no process by which such transformation might be effected, still less are we acquainted with any internal forces which can mould them into cells with functions to perform. On this account a very great deal of thoroughly sound experimental evidence is necessary to establish the doctrine of evolution of life *de novo*. But, so far as our present knowledge guides us, whether we term it spontaneous generation, abiogenesis, or archebiosis, the process by which living things spring from lifeless matter must be said to be only ideal.

Note.—In describing certain organisms as *mucors*, those thecasporous fungi are meant of which *Ascophora mucedo* is the type; they are described by Pasteur as *mucédinées*; and he seems to include in this description *Penicillium*, which is basidiosporous *. *Mucors* he classes as those vegetable organisms which take the form generally on the surface of a liquid of a thin pellicle with a more or less fatty or gelatinous appearance. Such matter I have noticed accompanying conferva. The fungus in chains found in urine is identical with that supposed by Pasteur † to cause the production of carbonate of ammonia from urea; while those small cellules without a nucleus, which I surmise, for a reason already stated, to be *Torula* in an early stage, he believes to be specifically different ‡. With regard to filters of nitrocellulose, I some time since wished to modify ordinary filters so as to make them more easily combustible in the crucible when used for quantitative analysis. This I tried by dipping them in strong nitric acid, washing in water, and finally in ammonia. As far as my recollection goes, I obtained a denser kind of paper, which answered the purpose very well. On endeavouring recently to make this variety of filter-paper, I met with the same difficulty which Prof. Bloxam tells me some years ago he encountered when attempting the same object, namely, the parchmentizing of the paper, so as to render it waterproof. Either I have not used the proper strength of acid necessary, or else my former experience rested on the formation of nitrate of ammonia within the fibres of the paper.

* Ann. de Chimie et de Phys. tom. lxiv. p. 47. † *Ibid.* p. 52. ‡ *Ibid.* p. 52.

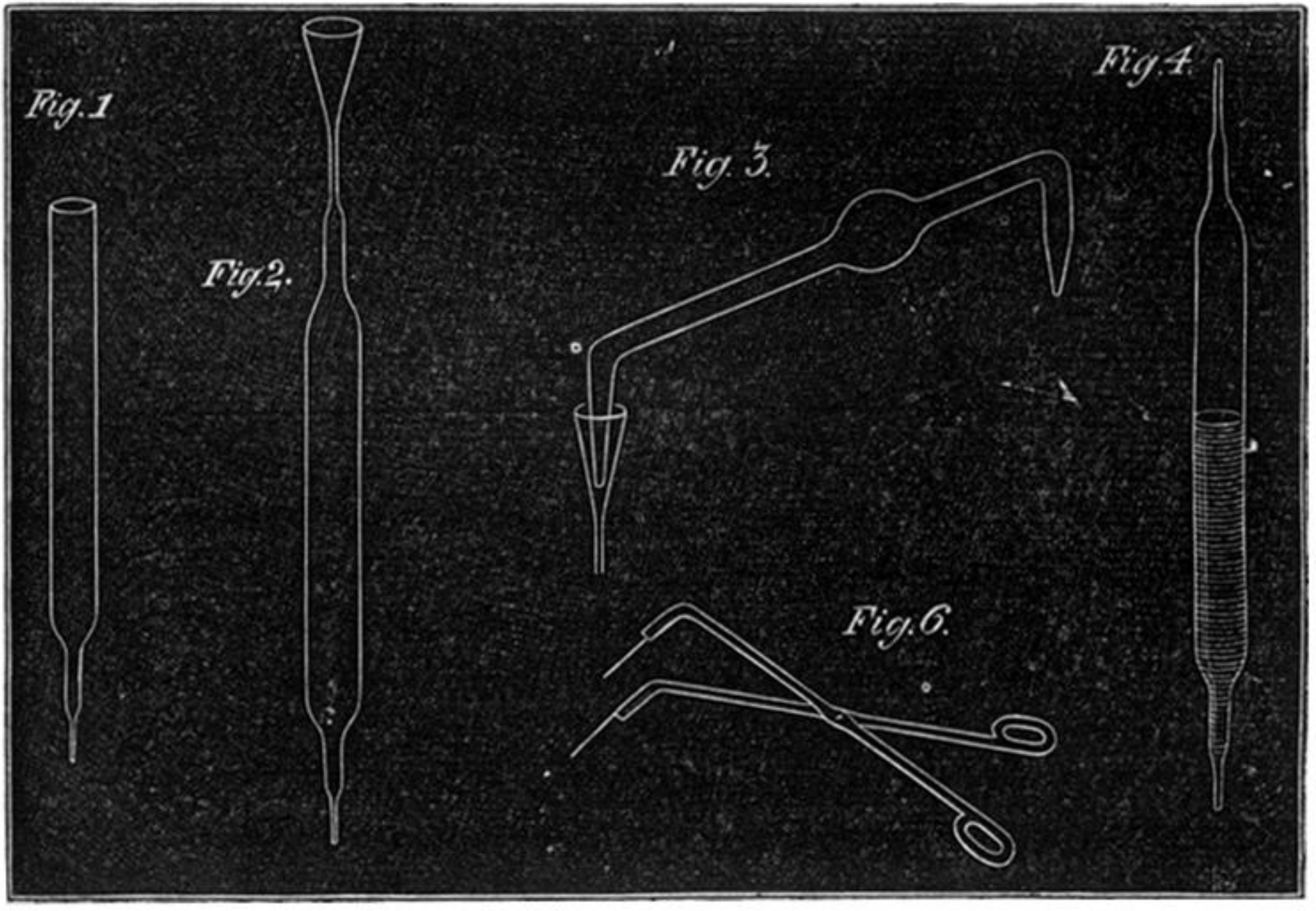


Fig. 1
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 6.

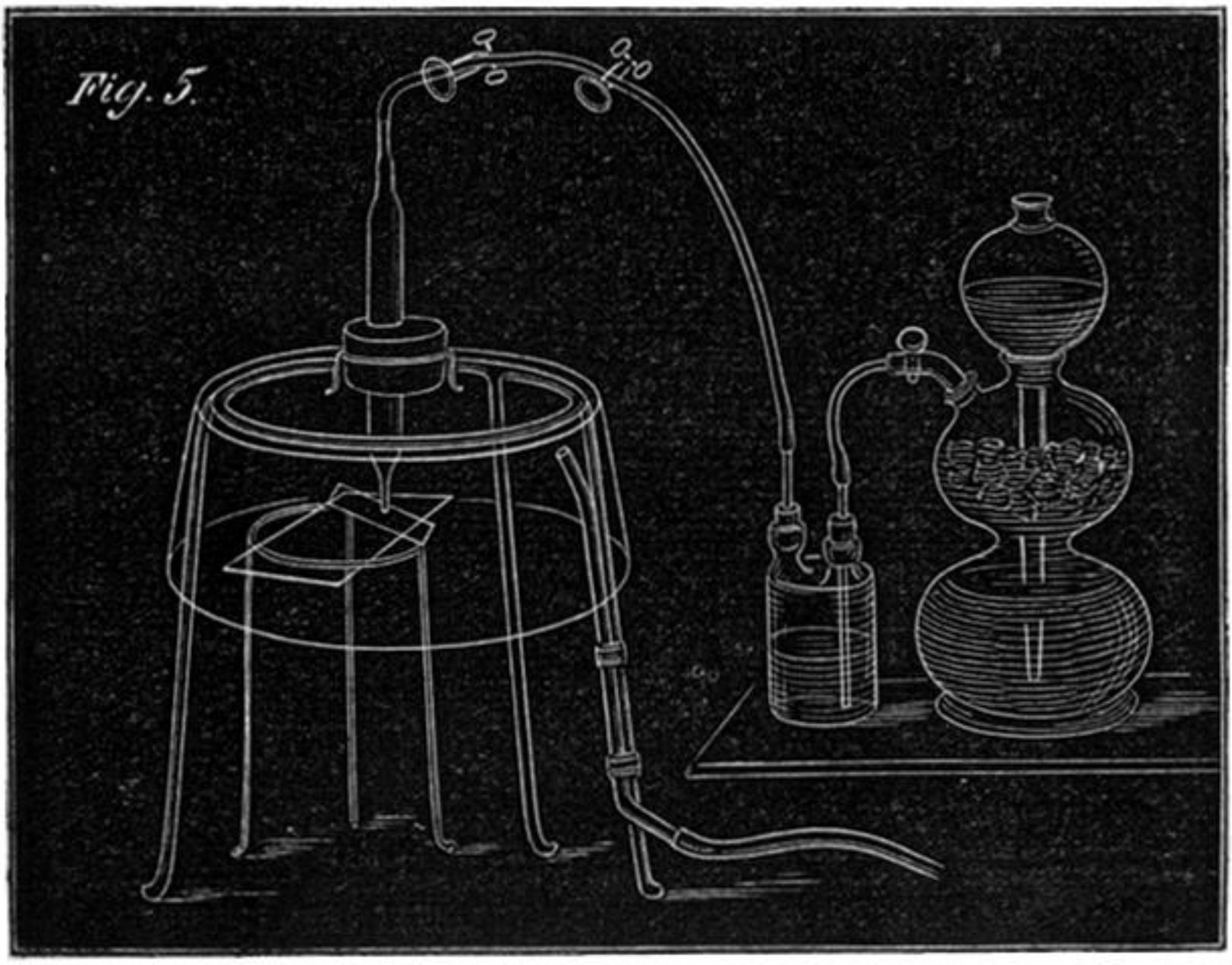


Fig. 5.