account of its intolerable stench. This experiment was repeated several times with similar results.

Schroeder and Dusch experimented in a similar manner with fresh sweet malt containing hops. After twenty-three days, the fluid being still unaltered, the cotton wool was removed, but the introduction of air—now unfiltered—was continued. The fluid was muddy and covered with fungi, and had undergone fermentation in a week.

These observers were, however, unable to obtain like results with milk, or with meat heated without the addition of water. These substances invariably underwent putrefaction.

In a paper published five years later Schroeder returns to this subject.[1] Having found that white of egg mixed with water, if constantly shaken while boiling, could be preserved for an indefinite time, he tried whether *ozone* had any power in inducing fermentation. Dilute sulphuric acid was decomposed by electricity, and the ozone thus generated was conducted into a vessel containing pure white of egg. The latter was kept for thirty-eight days, and was at the end of that time unaltered.

Schroeder was still unable to succeed with milk or yolk of egg, although the latter, if previously heated in a closed vessel in an oil bath to 160° C. (310° F.) generally remained unchanged, and the milk sometimes also kept pure.

The special constituents of milk could be easily preserved. He tested casein prepared by precipitation with acetic acid and then washing with water. The whey also which remained after this precipitation could be preserved with ease. When this whey was boiled a deposit occurred, and this was readily kept pure; and the whey which still remained did not ferment when preserved with the precautions mentioned.

Schroeder tried and succeeded with other materials, such as blood, urine, starch, &c.

The only substances which failed were milk, yolk of egg, meat heated without addition of water, and occasionally meat infusion.

As the result of these contradictory experiments he came to the conclusion that there were two ways in which fermentation might be caused; firstly, by some solid particle which

[1] *Annalen der Chemie und Pharmacie*, cix. 1859.

can be arrested by cotton wool, and, secondly, by oxygen gas (in the case of milk, yolk of egg, &c.).

Two years later there appeared another paper by Schroeder referring to those substances which he had previously failed to preserve, and in this research he has recourse to the use of higher temperatures than formerly.[1]

Yolk of egg, after being heated for half an hour in a closed glass vessel, at a temperature of 130° C. (266° F.), was placed in a flask the neck of which was stuffed when hot with cotton wool, and was again boiled with a little water. This remained for seventy days unchanged.

He succeeded in a similar manner with meat and milk, and in the case of the latter he found that prolonged boiling at 100° C. was sufficient.

From these facts he gives up his formerly expressed view as to the spontaneous fermentation of organic substances under the influence of oxygen, and concludes that in these fluids spores were present which could resist a boiling temperature, the development of these spores being, according to him, the cause of the fermentation. He further considers that these spores were present originally in the milk, and were not introduced from the air, because he finds that milk which has not been boiled at all putrefies sooner than pure boiled milk exposed to the air.

It may be interesting to mention here that similar difficulties were experienced by Appert in his attempts to preserve milk. He succeeded by the following method: 'Condense the milk to two-thirds of its volume, strain it, then put it in the bottle, seal and boil in a water bath for two hours.' In order to prevent the cream from separating he found it well to add yolk of egg. This did not increase the difficulty in preserving it.

Still further evidence disproving the gaseous theory is furnished by Pasteur.[2] He repeated Schwann's experiments and was successful with most fluids, but for a time he failed in the case of milk. He, however, succeeded when he boiled the milk under pressure at 110° C. (230° F.) for one or two minutes, heated air being then allowed to come in contact with it; and

[1] *Annalen der Chemie und Pharmacie*, cxvii. 1861.
[2] *Annales des Sciences Naturelles*, série iv. t. xvi. 1861: *Zoologie*

he also succeeded if he subjected the milk to prolonged boiling at 100° C. Such milk remains unaltered for an indefinite length of time, but it readily decomposes if unheated dust be introduced into it in the manner to be afterwards described.

The most striking of Pasteur's experiments is that of the flask with the bent neck. A flask containing, say, yeast water is heated so as to render its contents pure. Its neck is drawn out and bent, and then, after boiling, the lamp is simply with-

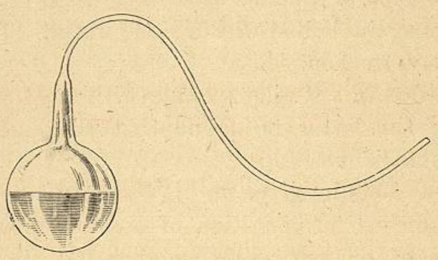

FIG. 3.—PASTEUR'S FLASK WITH THE BENT NECK.
(From Pasteur.)

drawn, the neck being neither heated, sealed, nor plugged (Fig. 3). Nevertheless the fluid does not undergo any change.

If, on the other hand, the neck of this flask be sealed during ebullition, a more or less perfect vacuum is thus produced, and then, if the neck be broken after cooling, air rushes violently into the flask, carrying with it its dust. The result is that fermentative changes occur in the fluid. In the same way, if one of the flasks with open necks, the contents of which have remained for some time pure, has the neck broken off short, the fluid in its interior rapidly undergoes fermentation; or again, if the neck be not bent but be kept straight, so as to allow dust to fall in, fermentation rapidly occurs.

The explanation of these results is that in the case of the flask with the long neck the dust is caught in the curve, which in the first inrush of air is filled with water, which filters the air (Mr. Lister's view). Pasteur had supposed that part of the air dust entered the vessel instantly, but that the fluid and the walls of the flask were at that time so hot that any living particles present were immediately destroyed.

As will be seen further on, Pasteur also found that it was

not necessary to filter the air of its dust, but that if the air were merely left undisturbed for some time till the dust settled, it might then be introduced into flasks without causing any development.

These experiments have been repeated by various observers with success, and Mr. Lister has at present in his possession a flask of this kind containing urine which is now thirteen years old, but which still remains unaltered and as limpid as on the day it was prepared.

In a lecture on Haze and Dust,[1] Professor Tyndall showed that if no dust were present in a flask, a beam of condensed light passed through the vessel in a dark room would only be visible on each side of it, but would be invisible in its interior; in other words, we see light only because there are particles in the air which render it visible. Were there no particles all would be darkness.

Such being the case, Tyndall found that when the air which was admitted to a flask had been previously heated, as in Schwann's experiment, the beam of light was not visible in the interior, showing that all or almost all the particles had been destroyed by heat, or, in other words, were in the main of an organic nature. By the same method Mr. Lister has found that in Pasteur's flasks with the long open necks, no floating dust is present after what was originally there has settled.

Another method of excluding dust was published in 1873 by Mr. Lister.[2] It seemed probable that the occasional failures which occurred in the attempts to preserve boiled fluids arose from the fact that the steam did not destroy the septic energy of the dust in the necks of the flasks which had not been previously heated. Mr. Lister, therefore, in addition to the precautions as to boiling under cotton-wool caps, &c., subjected his flasks to a high temperature previous to the introduction of the fluid. This is done by keeping them, after the cotton cap has been applied, in an iron box, which is kept at a high temperature for two hours.

This box is of a square form, with one of its sides movable so as

[1] *Nature*, Jan. 27, 1870.
[2] *Microscopical Journal* for October 1873; see also *Trans. of Path. Society of London*, vol. xxvii. 1878.

to form a door. 'This door has its circumferential part in the form of a groove capable of being packed with a considerable mass of cotton wool (Fig. 4, F). This door can be secured by means of nuts against the edge of the box; and the cotton wool, having the narrow rim of metal thus firmly pressed against it, serves as an effectual filter of the air that passes in during cooling. But then it is essential that the heat be so equally distributed as to avoid heating any portion of the cotton to such a degree as to destroy its physical properties. This uniformity of heat is provided for by having three shelves of sheet iron interposed between the large Bunsen's burner and the bottom of the box, so as to prevent the heat from acting directly upon it, while at the

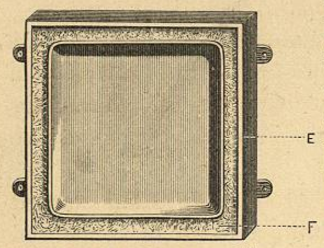

FIG. 4.—DOOR OF MR. LISTER'S BOX.

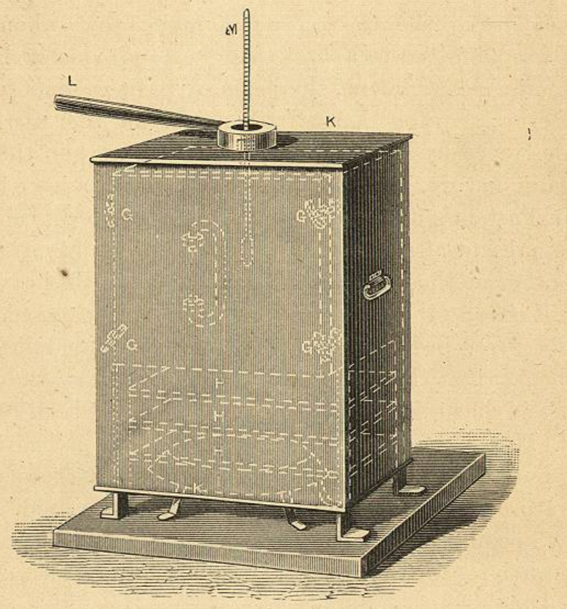

FIG. 5.—MR. LISTER'S HOT BOX.

same time the box is covered over with a cover of sheet iron (Fig. 5, K), which reaches nearly to the ground, and, while it checks radiation, compels the heated air to travel over the whole exterior of the box

and escape by holes at the top of the cover, whence it is conducted into a chimney by a tube (L). By these two means combined, the shelves below and the cover round about, we get a uniform browning of the cotton. Into such a box the requisite number of vessels are introduced (Fig. 5). An aperture in the top of the box well packed with cotton wool transmits a thermometer (M), to show us when the temperature of 300° F. has been reached, and when this, or any other higher degree short of 350° F., has been continued for two hours, the gas is turned off and cooling is allowed to take place; and when the apparatus is quite cool, the covered glasses may be removed with confidence that they are perfectly free from living organisms.'

FIG. 6.—MR. LISTER'S LARGE DOUBLE-NECKED FLASKS.

In this manner Mr. Lister purifies his flasks. The larger flasks have two necks, a large vertical one and a lateral one, which is a bent spout, large at its commencement and comparatively narrow at its shorter terminal part beyond the bend (Fig. 6, O). The large size of the first part prevents it from acting as a siphon, and the result is that when the liquid is poured from such a flask and the vessel is afterwards restored to the erect position, the end of the nozzle remains filled with a drop of the liquid, and this guards the orifice so that regurgitation of air can never take place through the nozzle. This drop of fluid being sucked away by means of a carbolised rag, a pure cotton cap is tied over the orifice, and the flask is kept for future use.

This flask, purified by heat and with each orifice covered with pure cotton caps, is used for the experiments (see Fig. 7). The fluid to be tested is introduced into it by means of a siphon, consisting of two glass tubes (S and T)connected by a tube of india-rubber (U), with a stop-cock (V) in the course of the india-rubber tubing. The siphon is first completely filled with water, the temperature of which should be higher than that of the air, so that there is no dissolved air given off to form bubbles. Place one leg of the siphon in the vessel containing the fluid to be used (W), then turn the tap and permit a sufficient amount of fluid to flow out to ensure that all the water has escaped from the siphon; then turn off the stop-cock, wash the outside of the tube (T) with carbolic lotion, wrap a mass of carbolised rag (Y) around its lower extremity, and apply this to the mouth of the flask (X) as soon as the cotton cap is removed; push the tube steadily down to the bottom of the flask through the carbolised rag, turn the stop-cock, and let the required amount of fluid flow into the flask (Fig. 7). When this has taken place the tap is again turned off, the

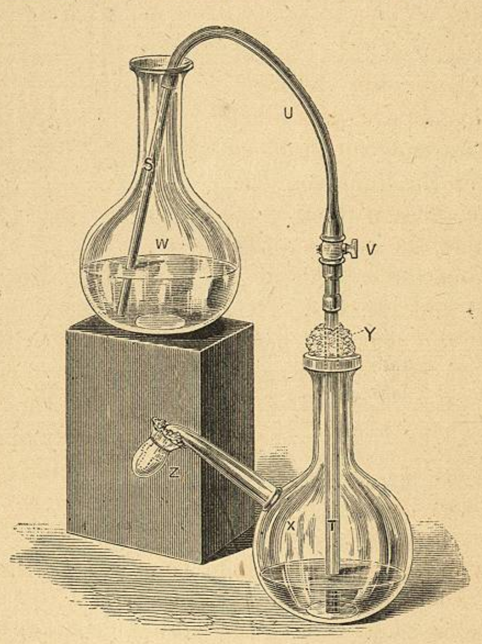

FIG. 7.—METHOD OF FILLING THE FLASKS.

siphon is withdrawn through the antiseptic rag, and a fresh cap of carbolised cotton (the cotton is carbolised by being soaked in a solution of one part of crystallised carbolic acid in one hundred parts of anhydrous ether and allowed to dry) is tied over the mouth of the flask when the rag is withdrawn. The fluid is now heated for the desired length of time, and then abandoned under the protection of the caps.

In this way Mr. Lister has found that he can preserve turnip infusion, hay infusion, urine, fresh milk, &c., for any length of time without any alteration taking place. To preserve milk, the flask containing it is immersed in boiling water for half an hour or more.

But this is not all, for these fluids can be transferred to smaller vessels without undergoing any fermentation after this transference. This is done as follows: a liqueur glass (A) is covered by a glass cap (B) (watch glass), and the whole by a glass shade (C), the liqueur glass and the shade standing on a glass plate (see Fig. 8). This arrangement is introduced into the hot box and thoroughly purified. Thus we have a pure glass filled with pure air, and the problem is to transfer the fluid from the flask to the glass without contamination in the process. To do this, the cotton cap is removed from the nozzle of the flask (Fig. 7, Z) and the end of this is instantly slipped into an opening in the centre of half an india-rubber ball (Fig. 9, R) previously steeped in a strong watery solution of carbolic acid. The outer glass shade is then removed, and the watch glass being lifted, the india-rubber cap is instantly applied in its place (see Fig. 9). The required quantity of fluid is then poured into the glass, and the cap and shade immediately reapplied. A fresh cotton cap is now tied over the nozzle of the flask. In this manner any number of glasses may be charged, and these are found to remain as pure and unaltered as the fluid in the original flask.

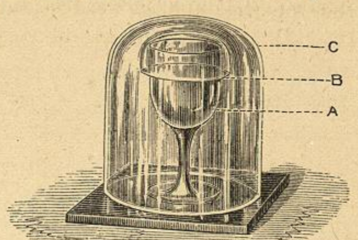

FIG. 8.

And now observe what such experiments teach. In the first place, into the original flask, when cooling, air enters, but this air having passed through a cotton-wool plug is incapable of causing putrefaction. Then in the decanting of this liquid from the flask, fresh air must enter through the large mouth of the flask, but as this passes through a filter of cotton wool it is in like manner incapable of causing fermentation. Further the liqueur glasses are full of air, which has either been previously heated, or which has been filtered

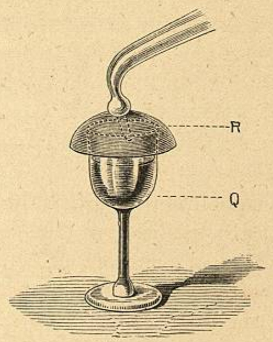

FIG. 9.

through the cotton wool around the door of the hot box. The fluid when poured from the flask into the glass mixes freely with this air, but no change is set up. And, lastly, the loosely-fitting glass cap and shade allow a free interchange of air, but are so placed as to make sure that the air deposits its dust outside the glass, thus corresponding in action to Pasteur's flasks with the bent necks. In spite of all these opportunities of admixture with the gases of the air, all sorts of fluids remain unaltered, while, on the other hand, the same liquids exposed freely to unfiltered air rapidly undergo fermentative changes.

These experiments are of themselves an absolute proof that the gases of the air alone are unable to cause fermentative changes in organic substances.

In 1874 Dr. Roberts[1] demonstrated again that fresh milk and other substances could be prevented from putrefying if kept in a flask with a cotton-wool plug after having been previously boiled.

His method was as follows: An ordinary delivery pipette, having on it an oblong bulb capable of containing 30–50 cc., was sealed at one end, and the materials to be experimented on were then introduced into the bulb until it was two-thirds full (Fig. 10, A). The inside of the neck of the bulb was next wiped dry, and a plug of cotton wool was inserted about its middle. Lastly, the neck was drawn out above the plug and sealed in the flame (Fig. 10, B).

When the bulb was thus charged and sealed it was weighted with a leaden collar, and submerged in the upright position (so as to prevent the wetting of the cotton-wool plug) in a can full of water. The can was placed over a source of heat and boiled for the required time. The bulb was then withdrawn and, when quite cold, its neck was filed off above the cotton-wool plug (Fig. 10, C). Finally it was set aside in the upright position and maintained at a suitable temperature.

By exposure to the heat of boiling water for from twenty to forty minutes Roberts was able to preserve those substances with which Schroeder and other observers had failed, viz., milk, pieces of meat, and egg albumin.

In 1876 experiments were published by Professor Tyndall[2]

[1] *Philosophical Transactions*, 1874.
[2] *Philosophical Transactions*, 1876.

which afford still further evidence on this subject. I have already mentioned the method by which he demonstrated the presence or absence of particles in suspension by passing a powerful beam of light through the air to be examined. He found that 'in order to render air optically pure it was only necessary to leave it to itself for a sufficient time in a closed chamber or in a suitably-closed vessel. The floating matter gradually attaches itself to the top and sides, or sinks to the bottom, leaving behind it air possessing no scattering power. Sent through such air the most concentrated beam fails to render its track visible.'

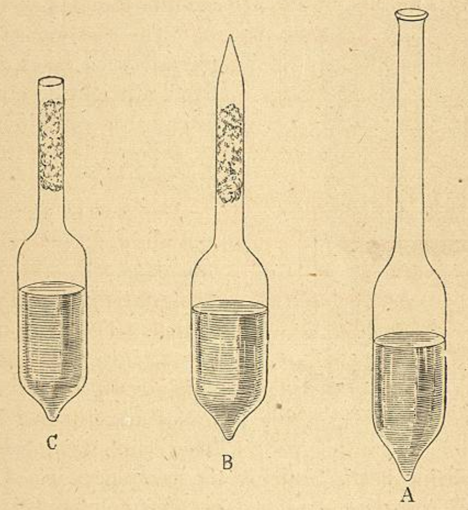

FIG. 10.—DR. ROBERTS' BULBS (COPIED FROM ROBERTS).

His method as described by himself is as follows: 'A chamber or case was constructed with a glass front, its top, bottom, back and sides being of wood. At the back is a little door which opens and closes on hinges, while into the sides are inserted two panes of glass facing each other. The walls of this case are smeared with glycerine in order to make the dust adhere. The top is perforated in the middle by a hole 2 inches in diameter, closed air-tight by a sheet of india-rubber. This sheet is pierced in the middle by a pin, and through the pinhole is passed the shank of a long pipette ending above in a small funnel. A circular tin collar 2 inches deep surrounds the pipette, the space between both being packed with cotton wool mois-