described, I have found that blood, removed from the healthy living body and placed in calcined flasks or in flasks containing infusion of cucumber, may be preserved for an indefinite length of time without alteration.

Hence blood has no inherent tendency to undergo fermentative changes, nor can oxygen alone induce such alterations.

III.—*Urine.*

Healthy urine was first preserved without alteration by Van der Broeck. The flasks in which it was received were prepared in the manner before described. An animal (dog or sheep) was killed, the abdomen was immediately cut open, and the ureters and urethra having been rapidly tied, the bladder was removed and immersed in the mercury. A heated needle was then introduced, and the bladder was torn, the urine then ascending into the glass. This urine remained pure even after the addition of oxygen or filtered air.

In the same paper in which Pasteur mentions that he has succeeded in preserving blood he states that he has also obtained pure urine. The method is described in his 'Etudes sur la Bière.' The flask with its nozzle and stop-cock are prepared as in the case of the blood; then the free extremity of the brass tube is introduced into the urethra. Urine being passed, the stop-cock is turned, and the urine flows into the flask. Urine thus obtained undergoes no fermentation. 'Elle dépose des cristaux en petite quantité, mais sans se troubler ni se putréfier d'aucune façon.'

In 1871 Mr. Lister succeeded in obtaining and preserving unboiled urine.[1] The method he employs is to wash the meatus urinarius and the glans penis with 1–40 carbolic lotion. A prepared flask is then taken, the cotton cap is removed, the glans immediately applied over the orifice, and urine passed into the flask. A fresh cotton cap is then applied. This urine may, like other fluids, be decanted into liqueur glasses. This experiment was apparently constantly successful, no alteration occurring in the urine in the flasks or in the glasses.

[1] *Transactions of the Royal Society of Edinburgh*, 1875.

I may here state that I have often repeated this experiment with the view of obtaining pure unboiled urine for other experiments, and always with success. I have, however, used the spray, and have thus avoided the necessity of applying the glans penis to the orifice of the flask. The glans having been purified, urine is simply passed in a spray of carbolic acid into a pure flask. This urine passed through the air, but that air, having been acted on by carbolic acid, was inert.

Dr. Roberts has also obtained similar results by passing urine into a pure test-tube, and afterwards charging tubes of the form previously described, by breaking off the capillary end below, and letting the urine flow up. Of eight tubes so obtained, the urine remained unaltered in seven, while in one it putrefied.

Cazeneuve and Livon[1] succeeded in preserving urine in the urinary bladder without the occurrence of any alteration in it.

A ligature was placed around the prepuce of a dog for five hours, in order to have a considerable amount of urine in the bladder. An incision being made into the abdominal cavity at the end of that time, the ureters and the urethra were ligatured, and the bladder was cut out. The bladder was then suspended in the air at a temperature of about 25° C. The wall of the bladder soon dries, and though liquid slowly transudes, that liquid evaporates immediately, and thus the bladder wall cannot putrefy. Urine may be kept thus for several days without undergoing any change, although if the bladder be opened it becomes ammoniacal in twenty-four hours. I shall return to these experiments at a later period.

Thus healthy unboiled urine has no inherent tendency to putrefy, but follows the same law in this respect as boiled urine.

IV.—*Milk.*

In 1859 Hoppe-Seyler attempted to preserve milk pure in the following manner:—[2]

A small funnel was carefully fastened over the teat of a goat. To the lower end of this was fastened a piece of caoutchouc tubing, the

[1] *Revue Mensuelle*, 1877, p. 733.
[2] Virchow's *Archiv.* xvii. (1859).

other end of which was attached to a glass tube below. This glass tube passed down to the bottom of a glass test tube, the upper rim of which was provided with a piece of caoutchouc tubing open above. None of the tubes were heated nor in any way purified. The milk was now withdrawn in a continuous stream, so as to flow for a long time over the edge of the caoutchouc tube till it was quite free from bubbles of air. The test tube was then lowered, and then, while the milk was still flowing, the caoutchouc tube was firmly tied around a thick glass rod.

Milk obtained in this manner, and kept at the ordinary temperature, coagulated in three days. Hoppe-Seyler therefore concluded that milk when shed contains a ferment.

This experiment proves that oxygen is not necessary for the occurrence of fermentation in milk; in other words, it is not the cause of such changes, and therefore, as the tubes were not purified, the cause must either be in the milk itself or be something adhering to the tubes. As I have just stated, Hoppe-Seyler concluded that the cause was inherent in the milk.

Which of these is the true agent is decided by the following experiments performed by Dr. Roberts:—[1]

'A glass tube was drawn out at each end to a narrow orifice. The lesser portion of this was tightly wrapped round with cotton wool and inserted as a plug into a large test tube containing water to the depth of one inch. A cap of cotton wool was also tied over the narrow orifice. The water in the test tube was then briskly boiled, and the boiling was continued almost to dryness. When the apparatus was cold I took it into the cowhouse, and seizing a teat, I pulled off quickly the cotton-wool cap and pushed the narrow point into the duct of the teat. Holding it firmly in this position I milked into the test tube until sufficient milk had been obtained. I then drew away the test tube from the little tube, pressing in the cotton wool around it as I did so, until the latter was entirely withdrawn from the test tube.

'From the test tube I charged ten empty pure tubes' (in the manner described under urine), 'and resealed their capillary orifices: of these ten tubes three remained unchanged, the milk remaining perfectly normal as regards taste, reaction, &c. The other tubes curdled or putrefied in ten days.'

[1] *Loc. cit.*

The method described here is imperfect, but the fact that three tubes remained unaltered absolutely demonstrates that the cause of the fermentation is nothing inherent in the milk itself, but something which it acquires after it leaves the body—that something being particulate, not gaseous.

Mr. Lister[1] describes several series of experiments performed with the same aim. In one of these he succeeded in preserving the milk unaltered.

A number of little tubes were covered with glass caps and shades, and purified in the usual manner. After a rainy day he washed the udder of a cow and the hands of the milkman with water. A wide glass tube connected with an elastic tube was then placed under the nipple (the glass tube had been heated and the elastic tube boiled). This was filled with milk, and then each little tube in succession had a small quantity introduced by relaxing the elastic tube.

Of twenty-four tubes so prepared and charged two remained permanently pure. The results in the other tubes equally demonstrated that the cause of the fermentation of milk is not inherent in the milk, for the milk in each underwent a different change. These experiments will be more fully considered at a later period.

While in the Shetland Islands in the summer of 1880 I performed a series of experiments, which consisted in obtaining the milk under the protection of a spray of carbolic acid. A number of flasks with cotton caps and long necks were heated before leaving London. The udder of the cow and the hands of the milkmaid being washed with carbolic lotion (1–20), the flasks were uncorked and filled with milk under the spray. In doing so the mouth of the flask was held as close as possible to the teat. The cow was restive and would not allow me to do the milking, and therefore the experiment was performed by the milkmaid. When the restiveness of the cow, the inexperience of the milkmaid at antiseptic work, and the dark and draughty cowhouse are taken into account, it will not be surprising that the milk in a considerable number of the flasks fermented; but nevertheless evidence was got, of the same kind as that obtained by Dr. Roberts and Mr. Lister, sufficient

[1] *Microscopical Journal*, 1878.

to disprove the existence of a ferment in the milk when withdrawn from the body.

In order to transfer these flasks to London I had intended to draw out and seal their necks, but I found this impossible, and therefore I soaked pieces of cork in carbolic lotion, inserted them into the mouth of the flask, and covered them with tar —a very inefficient method. During the voyage the milk was much shaken, and some of the corks proved inefficient, as shown by the leakage of the milk.

The following are the experiments, with their results :—

First Experiment.

August 5, 1880.—The udder and teats of the cow and the hands of the milkmaid having been washed with 1–20 carbolic lotion, and a small spray being directed as well as possible over the part, eight purified long-necked flasks were filled with milk, the milk being drawn directly into each flask, which were held as near the teats as possible. Eash flask was re-covered with its cotton cap, and they were then placed in the upright position in a warm room.

August 8.—The milk in these flasks seems unaltered. There is a little cream on the top in each.

August 10.—Ditto.

August 24.—Four of the flasks have undergone change, the change varying in nature in each flask. The other four are perfectly fluid and present the appearance of pure milk.

To-day the corks were inserted.

September 21 (twenty-three days after the transport to London).—Only two flasks now remain pure, the other six having undergone alterations of various kinds.

October 27.—Examined. The milk in two flasks is perfectly normal.

The result of the first experiment was, that after nineteen days four of the milks had undergone alterations of various kinds, while four remained apparently pure. On October 27—*i.e.* after two months and twenty-two days—two milks were still perfectly right, in spite of a sea voyage and great disturbance.

Second Experiment.

(*a*) *August* 10.—Two flasks were filled after washing the udder of the cow and the milkmaid's hands with carbolic acid (1–20). No spray used.

August 24.—The milk in one of these flasks has undergone alterations; the milk in the other is perfectly pure.

September 21.—Both milks have coagulated and are undergoing changes.

(*b*) *August* 10.—After the spray employed in experiment (*c*) had been stopped, two flasks were filled without it.

August 24.—I am doubtful whether these are pure or not; I think they are not.

September 21.—Changes have occurred in both.

(*c*) *August* 10.—Seven flasks were filled under the spray as usual.

August 24.—Three of these milks have altered; four are still apparently pure.

September 21.—Three still remain apparently pure: four have undergone change.

October 27.—Examined. Three still pure.

On December 24 I opened one of these flasks, and found a slightly suety smell but a perfectly sweet taste, and the milk presented the appearance of normal milk. I examined it microscopically—no organisms. I have stained some specimens of this milk (Plate V. Fig. 33), and it will be seen that no organisms whatever are present, and this in unboiled milk kept for more than five months.

The result here is that three out of seven of the flasks filled under the spray have remained permanently pure, while all of those filled without the spray have ultimately undergone fermentative changes.

Third Experiment.

(*a*) *August* 16.—One flask filled without spray and without previous application of carbolic acid to the hands or teats.

August 24.—This milk has coagulated.

(*b*) *August* 16.—One flask filled without the spray and without washing the teats with carbolic acid. The hands of the milkmaid, were, however, purified.

August 24.—This milk has undergone fermentation, having separated into two layers—the upper clear, the lower thick but not coagulated.

(*c*) *August* 16.—Two flasks were filled without the spray, but after washing both the teats and the hands with carbolic lotion.

August 24.—One of these is doubtful, the other apparently unaltered.

September 21.—Fermentative changes are occurring in both.

(*d*) *August* 16.—Six flasks filled under the spray as usual.

August 24.—All these are apparently unchanged.

September 21.—Four have undergone some fermentative changes. One is doubtful. One is still pure. Three of the corks have not fitted perfectly.

October 27.—Examined. One still pure.

The whole result is, that of twenty-one flasks filled under the spray, six remained permanently unaltered, and that after having been exposed in a manner which sufficiently explained the occurrence of fermentation in some of the others.

Up till August 24th no less than fourteen of these milks had remained apparently unchanged, while similar specimens taken without any precautions had undergone alteration.

I have still in my possession (June 1881) four of these six flasks, and the milk in these still remains perfectly pure and free from fermentative changes.

From all the facts narrated I think it is absolutely certain that milk has no inherent tendency to undergo fermentation of any kind, and that the cause of the fermentation is not the gases of the air, but solid particles which the milk meets with after it is drawn from the cow.

V.—*Egg Albumen.*

The difficulty experienced by Schroeder in preserving boiled white and yolk of eggs will be remembered.

Van der Broeck introduced an egg into the mercury arranged as formerly described, broke the shell with a heated iron rod, stirred up the contents with a similar rod, and then allowed them to ascend into the glass. This egg albumen remained pure, even after subsequent addition of oxygen or of filtered air.

Gayon[1] found that some eggs may be preserved unaltered, while others undergo change. He supposes that in the latter

[1] *Comptes Rendus*, lxxvi. lxxvii.

case the causes of putrefaction entered as the egg passed through the oviduct. Such an idea is, however, hardly tenable.

Roberts has shown by experiments similar to those previously described that egg albumen has no inherent tendency to undergo fermentation.

He proceeded in the following manner:—Eight sterilised tubes were prepared containing pure water. 'A fresh egg was fixed in a convenient support, and a small piece of the shell was chipped off, care being taken to leave the subjacent membrane uninjured; then a sterilised bulb was taken, and the capillary portion immersed for a few seconds in boiling water, in order to destroy any adherent septic particles. The sealed end was then rapidly snipped off and the capillary portion plunged into the interior of the egg. About 2 gm. of the albumen were then sucked up by the mouth into the bulb. When this was accomplished the bulb was quickly withdrawn and its capillary end sealed in the flame.'

Six of these eight tubes remained unaltered for seven months.

Of a second series of seven tubes similarly charged and kept for two months, five remained unaltered. That is, of fifteen tubes filled, eleven remained pure.

I may refer to an experiment which I did for another purpose, accepting as true the view that egg albumen had no inherent tendency to undergo fermentation, and which proves the truth of that view.

On July 7th, 1880, I took four purified beakers and four fresh eggs. These eggs were washed with carbolic lotion (1–20), and were then broken, one into each beaker, under the spray.

One of these beakers was covered with its cotton cap, and placed in an incubator kept at the temperature of 98° F.

On July 20th no change whatever had occurred. The other flasks were used at once for various experiments. Into one a special form of organism was introduced, and here only this one form of organism developed, with the production only of a special kind of fermentation.

Hence egg albumen has no inherent tendency to undergo fermentative changes.

VI.—*Vegetable Tissues.*

Dr. Roberts has also experimented on the solid tissues of the turnip, potato, orange, and tomato, with similar success.

The following is his method for turnip :—

'A sterilised tube containing water was nicked with a file near the base of the capillary part, where the tube had a diameter of about two millimetres. A fresh oblong turnip was then fractured across, and the tube, snipped off at the nicked point, was quickly thrust into the substance of the turnip. A narrow cylinder of turnip about an inch long was thus forced into the column of water in the tube. The tube was then detached, and its end sealed with melted sealing-wax.' Of

14	tubes	thus charged	with turnip	10	were successful;
7	„	„	potatoes	4	„
8	„	„	orange	8	„
3	„	„	tomato	3	„

Ferments which induce changes after death are therefore not present in living vegetable tissues.

VII.—*Animal Tissues.*

Some years ago experiments were made by Billroth[1] and Tiegel[2] with the view of ascertaining whether the living tissues did or did not contain the causes of putrefaction. Having killed an animal, they opened its body rapidly, and removed with heated implements various portions of tissue such as liver, spleen, kidney, &c., and immediately dropped this into heated paraffin. They supposed that by this means any dust which fell on the tissue in its transit from the body to the flask would be destroyed by the hot paraffin, while this heat would not penetrate into and act on the interior of the tissue. At the same time the organs would be protected from air or dust by the paraffin.

They found that many portions of the body preserved in this way, notably the liver and spleen, underwent putrefaction rapidly, and they therefore concluded that the causes of this putrefaction were present in the living blood and tissues.

[1] *Coccobacteria septica.* [2] Virchow's *Archiv.* lx.

These experiments were repeated by Dr. Burdon-Sanderson, who obtained similar results and adopted the same views.

If, however, we look at the method, we shall find several objections to it. Thus, heated paraffin must be looked on as dry heat; it does not moisten solid particles in contact with it. Now it has been shown that dust, if kept dry, may be heated even to 300° F. without losing its power of causing fermentation. Further, paraffin solidifies at about 136° F., or even lower, and therefore paraffin, merely at its melting point, is not likely to be hot enough to destroy all septic particles. Further, during the cooling of the paraffin heavy particles of dust may fall into it and sink on to the tissue. Then, also, on the sides and bottom of the vessel is coarser dust, which likewise may not be destroyed.

But, again, paraffin is very apt to crack, and after cooling small cracks may occur which admit moisture and dust. To obviate this risk the paraffin has been covered with oil; but even here the oil becomes laden with dust and passes down through the cracks.

And, lastly, the knife, before dividing the tissue, compresses the vessels and forces the blood out of them, and thus, when these vessels are cut, air is sucked in, and this air carries its dust with it quite out of reach of the heat of the paraffin.

In December 1877 I commenced a series of experiments on this subject, and these have been continued at intervals since that time.

The first experiment was an imitation of those of Billroth and Tiegel (only it was performed antiseptically), and yielded conflicting results. Thus the liver and kidney putrefied, while the spleen, muscle, and mesentery remained unaltered.

This being the case, I determined to abandon this method entirely, and to see if some definite conclusion might not be arrived at in some other way. The following is a description of the method I have employed:—

A number of beakers, each provided with a cotton cap, were purified by heat, somewhat after Mr. Lister's method, and into each vessel about one-fourth of its volume of pure turnip infusion was introduced from one of the double-necked flasks (Fig 6, p. 19). This was done under the spray, and the cotton caps were then reapplied. These beakers