CHAPTER II.

THE PARTICULATE THEORY OF FERMENTATION (*continued*).—ON THE FERMENTATION OF UNBOILED SUBSTANCES.

Grape juice—*Gay-Lussac—Van der Broeck—Pasteur—Roberts*; blood—*Van der Broeck—Pasteur—Burdon-Sanderson—Lister—My own results*; unboiled urine—*Van der Broeck—Pasteur—Lister—My own results—Roberts—Cazeneuve and Livon*; milk—*Hoppe-Seyler—Roberts—Lister—My own experiments*; egg albumen—*Van der Broeck—Gayon—Roberts—My own experiments*; vegetable tissues—*Roberts*; animal tissues—*Billroth—Tiegel—Burdon-Sanderson—My own experiments—Chiene and Ewart—Meissner*—General review of the facts—Behaviour of similar fluids and tissues in the living body—Principles of aseptic surgery.

WHILE it cannot be doubted that the causes of the fermentation of *boiled fluids and tissues* are particles which reach them from the air and from surrounding objects, is this equally the case with the *unboiled*? Experiments with these substances are apt to yield very contradictory results, for it is a matter of extreme difficulty to prevent their contamination after their removal from the living body. How this has been managed and with what results we must now enquire.

I.—*Grape Juice.*

Gay-Lussac in the research mentioned before, attempted to ascertain whether unboiled grape juice remained unaltered when oxygen gas was excluded. He took a bell jar and introduced into it small grapes, still intact. The jar was now reversed over mercury, and was filled five times with hydrogen gas in order to wash out all the oxygen. The grapes were then crushed by means of an instrument introduced through the mercury, and the juice thus obtained was kept at a temperature of 15° to 20° C. Fifteen days later, no fermentation having taken place, a

small quantity of oxygen was introduced, and immediately fermentation commenced.

From these experiments he concludes that the oxygen introduced caused the fermentation. But here there are two main fallacies. In the first place, the skins of the grape were left mixed with the grape juice, no sufficient means being taken to destroy any solid particles adhering to them; and then also the oxygen introduced might have carried in the necessary particles. There can indeed be no doubt, from Pasteur's subsequent investigations, that the Torula cerevisiæ—the cause of the alcoholic fermentation—was present on the skins of the grapes; and Pasteur has further shown that oxygen is absolutely necessary for the development of the old cells of the Torula, though the young cells may go on developing without the presence of free oxygen. The explanation of Gay-Lussac's experiment is, therefore, that the old Torula cells present could not develop without oxygen, but that when a small quantity of oxygen was introduced, they developed, and fermentation occurred.

The next attempt to preserve grape juice of which I can find any record was made by Van der Broeck, and narrated to the 'Provincial Gesellschaft für Kunst und Wissenschaft,' Utrecht, January 1858. His method was the following[1]:—

> Small beakers were filled with mercury, and then heated in a sand bath till the boiling point of the mercury was almost reached. From time to time they were placed under the receiver of an air-pump, and at the same time shaken in order to detach any bubbles of gas adhering to the side of the flask. This process of heating and exhausting was continued till all the air was removed from the bottom or sides of the glass. These glasses were then inverted in a basin containing previously heated mercury, and were firmly fixed in this position. Ripe and uninjured grapes were now passed into the mercury and brought under the orifice of the flask, a portion of the skin of the grape was clipped out by a heated knife, and by gentle pressure some of the juice was made to ascend in the vessel, the rest of the grape being removed. When a sufficient quantity of juice had been thus introduced the vessels were placed in a room of which the temperature was 25° to 28° C., and grape juice thus obtained could be kept for months or years without undergoing any change.

[1] See *Annalen der Chemie und Pharmacie*, cxv. 1860.

In this experiment not only was all air excluded, but the dust adhering to the walls of the vessel and in the mercury was subjected to strong heat, and its fermentative power destroyed. The juice of the grape, in ascending through the mercury, did not come in contact with unheated dust, nor did it touch the skin of the grape.

Into some of the flasks containing pure grape juice obtained in this way, pure and fresh oxygen was introduced from a retort containing chlorate of potash and oxide of copper. (The nozzle of the flask was heated previously to its immersion in the mercury, and the oxygen was allowed to stream out for a time sufficient to wash out all the dust.) In none of these flasks was there a trace of fermentation. Into others, atmospheric air, passed through a mass of cotton wool, was introduced in the same manner, but without producing any effect.

Into these vessels containing oxygen, yeast was introduced in minute quantity, and fermentation at once commenced. Into others containing only grape juice, young cells which had never been exposed to free oxygen were introduced by a method which is fully described in his research, and these also caused fermentation; thus proving that oxygen is not necessary even for the commencement of the change, if only the yeast cells be young (three or four days old).

By these experiments it was absolutely demonstrated—1 That oxygen is not the cause of the fermentation of unboiled grape juice; and 2. That the juice itself contains no ferment.

That grape juice contains no ferment was further shown by Pasteur,[1] who introduced unboiled juice into his flasks with bent necks, containing pure boiled juice. No fermentation occurred, though, as he says, if a single Torula cell had been added, the whole mass would have fermented.

Dr. Roberts[2] likewise succeeded with grape juice.

Test tubes were drawn out at their lower ends to capillary points and sealed in the flame; the upper ends were plugged with cotton wool; they were then passed and repassed through the flame of a spirit lamp until they were quite hot, as shown by the commencing charring of the cotton. (Fig. 12.)

'Eleven sterilised tubes, six empty and five containing water, were

[1] *Etudes sur la Bière.*

[2] *Phil. Transactions*, 1874.

charged with grape juice in the following manner:—A fresh grape was firmly seized with the finger and thumb, and a spot on its surface was pressed for a few seconds against the flame of a spirit lamp so as to destroy any adhering germs. The point of the sterilised tube, also heated in the flame and quickly snipped off by an assistant, was then thrust into the grape at the heated spot. Compression was now made on the grape until a sufficient quantity of the turbid juice was forced into the tube. The tube was then withdrawn, and its point sealed in the flame. The eleven tubes thus charged remained permanently unchanged, and when examined, at various periods from five to eight weeks, the taste and reaction of their contents were undistinguishable from that of the fresh grape juice.'

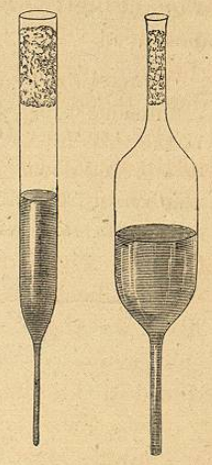

FIG. 12 (FROM ROBERTS).

II.—*Blood.*

Blood is one of the substances which have been frequently referred to as having an inherent tendency to decompose, but several experiments have now demonstrated that this is not the case.

The first observer who succeeded in preserving blood was Van der Broeck.

Van der Broeck proceeded as follows:—Having prepared his beakers filled with mercury as formerly described, he introduced one end of a previously heated copper tube into the carotid artery of a dog. To the other end of this a caoutchouc tube was connected, while the free end of the latter dipped into the mercury and the blood passed along it into the purified beakers. (This caoutchouc tube had been purified by the passage of steam through it for some time, and by placing a plug of cotton wool in each end while it was cooling.) The vessels were then kept at a temperature of 25° to 30° C. for weeks without the contained blood undergoing any change.

Into some of these flasks oxygen or filtered air was introduced, but still there was no putrefaction. The minutest portion of putrescent or even non-putrescent but unheated substance at once set up fermentation.

In 1863, Pasteur[1] stated that he had obtained blood from

[1] *Comptes Rendus*, lvi. 738.

healthy animals by means preventing contamination with unheated atmospheric dust, and that this blood had remained free from change. In a later publication [1] he describes the method pursued.

'For this I made use of a flask connected by means of a caoutchouc tube with a brass tube and stop-cock. The two parts of the tube are about twelve centimetres in length; that which is free is filed down like the extremity of a canula. In order to cleanse this vessel from all living dust the free extremity of the brass tube was connected with a platinum tube strongly heated, a small

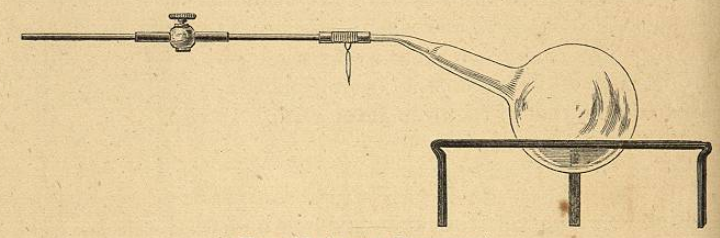

FIG. 13 (FROM PASTEUR).

quantity of water having been previously introduced into the flask. This water is then boiled, and the flask allowed to cool, the air which enters during cooling being previously heated. It is well to boil the water in the flask under pressure, to effect which the free extremity of the platinum tube is connected with a glass tube bent at right angles, which dips into a deep vessel filled with mercury. After boiling for some time under pressure, this tube is detached, and boiling is continued at the ordinary pressure; then the flask is allowed to cool and to become filled with heated air. When the flask is cold the cock is shut and the platinum tube detached. Till it is required it is well to hold the orifice of the brass tube down, in order to prevent dust from falling into it. Before being used this portion is heated carefully in the flame of a spirit lamp.

'A vein or artery of a dog is now opened, the end of the brass tube introduced, and secured in the blood-vessel by a ligature; the cock is then turned on. Blood flows into the flask, and when enough has been obtained the cock is shut and the flask placed in a suitable temperature.'

As a result this blood does not putrefy, and its odour remains quite fresh. There is not even an active absorption of

[1] *Etudes sur la Bière*, 1876.

oxygen, for after several weeks only 2 or 3 per cent. of that gas was found to have disappeared in a vessel sealed immediately after the blood had been introduced.

Dr. Burdon-Sanderson [1] also found that blood taken from rabbits with suitable precautions, and put into purified flasks covered with cotton wool, remained free from change.

Dr. Roberts,[2] having purified his tubes in the way described, and having thoroughly cleansed his finger, punctured it, and sucked up about two drops into each tube. Of ten tubes prepared in this way, six remained unaltered. This experiment is of little value, partly on account of the imperfect method of experimentation, and partly on account of the small amount of blood obtained.

Mr. Lister [3] took blood from the jugular vein of an ox in the following manner:—

A large glass tube was fixed in the large orifice of one of his double-necked flasks, the interval between the flask and the tube being filled with tightly-packed cotton wool. Over the outer end of this glass tube a cotton cap was applied, and there was a cotton cap as usual over the orifice of the spout. The flask thus arranged was heated in the hot box. The jugular vein of an ox having been exposed antiseptically, was divided, the cotton cap removed from the end of the tube, and the end of the vein slipped over the orifice of the tube. Blood thus flowed through a pure tube into a pure flask. When enough had been obtained the vein was removed and a pure cotton cap immediately applied in its stead. Before coagulation had occurred, various liqueur glasses, arranged as formerly described, were charged from the large flask.

Blood so obtained remained unaltered in the liqueur glasses and in the flask, though kept for six weeks.

Mr. Lister also found that not only blood, but blood and water—a much more putrescible mixture—remained unaltered. (The water was introduced into a large pure flask, and boiled so as to purify it. A portion of blood clot from one of the liqueur glasses was then spooned into the flask, careful precautions being taken against the entrance of living dust.)

In some experiments, performed in a manner to be shortly

[1] *Quarterly Journal of Microscopical Science*, xi. 1871.
[2] *Loc. cit.*
[3] *Microscopical Journal*, 1878.

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).