had been boiled. These were kept for some time till it was certain that the milk had been rendered sterile. Having calculated how many of these oval organisms were present in a given quantity of fermenting milk, he diluted this milk so as to have only one bacterium in a definite quantity (*e.g.* $\frac{1}{100}$th of a minim) of the fluid, supposing that the bacteria were equally diffused throughout it.

This was done in the following manner :—'By means of the syringe already described' (one graduated to the $\frac{1}{100}$th of a minim) 'one or more hundredths of a minim could be measured with precise accuracy; and I found that $\frac{1}{50}$th minim exactly occupied a circular plate of thin covering glass, half an inch in diameter, so that when such a drop was placed on a glass slide, and a cover glass of the size mentioned and quite flat was put down upon it, all air was expelled from under the latter, and the rim of fluid that formed round about its margin was so narrow as not to measure a quarter of the diameter of the field of the microscope even when the highest magnifying power was used. In other words, $\frac{1}{50}$th minim was disposed in a thin uniform layer of the exact size of the cover glass. Hence the number of bacteria under the glass slip—that is to say, in $\frac{1}{50}$th minim—was equal to the number of the bacteria in a field of the miscroscope multiplied by the number of times the area of that field went into the area of the covering glass. The micrometer gave the diameter of the field in thousandths of an inch; and the cover glass measured 500 thousandths of an inch across; and the areas of the circles were of course proportioned to the squares of those diameters. All that was needful, therefore, in order to enable me to calculate the number of bacteria in $\frac{1}{50}$th minim, was to form a fair estimate of the number of bacteria per field, and this was done by counting the organisms in a considerable number of fields, and taking the average.

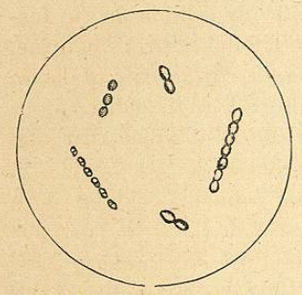

FIG. 71.—BACTERIUM LACTIS IN PAIRS AND CHAINS.

In one chain the component cells are undergoing division. (After Lister.)

'As the result of the estimate which I made of the number of bacteria present in every $\frac{1}{50}$th minim, I found it necessary to dilute the milk with no less than a million parts of boiled water, in order that every $\frac{1}{100}$th minim should contain on the average a single bacterium.'

Having obtained the necessary dilution Mr. Lister proceeded as follows :—'One-hundredth minim of the infected water was added by means of the syringe to each of five glasses of pure boiled milk. The result of this inoculation was that only one of the five glasses was affected at all.' The others remained unchanged, without fermentation, and without bacteric development. The one which was affected underwent lactic fermentation, and in it the bacterium lactis alone was found, no other form of organism was present. This bacterium was inoculated into urine and developed there. After four days milk was inoculated from this urine. The milk underwent lactic fermentation, and these bacteria were again found. Drops of urine, diluted so as to contain three bacteria per drop, caused lactic fermentation in all the vessels to which they were added.

The following experiments afford absolute proof that the bacterium was the cause of the fermentation :—

'On August 30 last (1877), having provided sixteen pure glasses of boiled milk, and having estimated, in the manner already described, the number of bacteria present in every $\frac{1}{50}$th minim of a glass of boiled milk, which had been inoculated the day before by touching it with a heated needle dipped in milk curdled under the influence of the pure ferment, I diluted a drop of this milk with boiled water to the requisite degree, and introduced into each of ten of the sixteen uncontaminated glasses a drop calculated to contain on the average a single bacterium, while five of the rest received each a drop supposed to contain two of the organisms, and the remaining glass was inoculated with a quantity in which, according to the estimate, there would be four bacteria. The result was that within three and a half days the glass into which four bacteria were supposed to have been introduced contained a curdled mass, and the five which had received the drops arranged for two bacteria each had all undergone a similar change. Of the ten inoculated with drops averaging one bacterium each the majority were at this period still fluid, but some assumed the solid condition in the course of the next twenty-four hours, though at different times. But of this series of ten, exactly five, as it so happened, remained permanently fluid.'

Every glass in which curdling had occurred contained the

bacterium lactis; the five glasses in which the milk was unaffected contained no organisms.

Hence it seems clear that when this organism is present in milk lactic fermentation occurs. Where it is absent this change does not take place, for, as Mr. Lister argues, we could hardly suppose that an organic molecule or ferment would occur exactly in the same cases as the organisms appeared, unless there was some intimate relation between them. If organic molecules, independently of the organisms, were the cause of this fermentation, some flasks ought to undergo lactic fermentation without the presence of any organisms; others ought to show development of these organisms, but no lactic fermentation.

Other Fermentations, especially the Putrefactive.

I may just refer more as a matter of historical interest than of real use in this question to Lemaire's experiments with carbolic acid and his opinions on fermentation.[1]

Lemaire showed that the addition of carbolic acid to organic fluids and tissues prevented putrefaction and other fermentations. Carbolic acid, according to him, did not interfere with the fermentations caused by 'unformed' ferments, such as synaptase, &c.

He then pointed out that the unformed ferments can act at temperatures at which the other ferments are inert, as, for instance, at zero and at 70° C. Trituration of yeast destroys its fermenting power, while trituration of emulsin does no harm. In, fact anything that favours life favours alcoholic and allied fermentations, while anything which is inimical to life is also inimical to these fermentations, though many of these things do not interfere with the action of 'unformed' ferments.

I have before referred to the experiments of Cazeneuve and Livon on unboiled urine.

The method of obtaining the bladder with its contained urine has been previously described; and in the successful experiments formerly mentioned on p. 37, no organisms were

[1] *L'acide phénique*, 1865.

found, while if the somewhat concentrated urine were removed and diluted with ordinary water it became alkaline in twenty-four hours, and filled with 'torulacée.' Results similar to those mentioned were obtained when the urine had been previously rendered alkaline by the administration of soda or potash.

Puncture of the bladder was soon followed by alkalinity and development of organisms in the urine: hence it is not the absence of oxygen from the urine which is the cause of the absence of change in it. The following experiment shows that the merest trace of oxygen is all that is required, if indeed it be at all necessary.

Prevent the evaporation through the walls of the bladder, by immersing it, immediately on its removal from the body, in melted paraffin at the temperature of 45° C. This temperature is insufficient to destroy the germs which fell on the wall of the bladder during its transit from the abdomen to the paraffin.[1] Thus a layer of paraffin covers the outside of the bladder, preventing the rapid evaporation of the fluid which exudes while living organisms are present on the wall of the bladder. In twenty-four hours remove the paraffin case. It is then found to contain an alkaline turbid fluid full of organisms. These organisms have not, however, had time in twenty-four hours to penetrate into the interior of the bladder, and therefore the urine inside is found to be still acid and devoid of life. *The same is the case with urine rendered alkaline*—the fluid outside contains organisms, that inside is free.

But let the bladder be first dipped in paraffin at 100° C., so as to destroy any living organism in contact with the wall, and then, after removing it from this paraffin at the end of a minute, let it be plunged into paraffin at 45° C., so as to get a thicker coat (this paraffin is previously heated to 110° C., and during cooling is protected from the dust), it will be found that even after three days the fluid outside the bladder—in the paraffin cup—is still clear, acid, and devoid of organisms. Leave this bladder now exposed to the air for say five hours, then give it a new coating of paraffin at 45° C., and leave this on

[1] The reason why the organisms do not develop on the bladder hung up in the air is that the fluid dries as soon as it exudes, and therefore the organisms have no fluid in which to develop.

for three days. The fluid outside the bladder will be found in this case to be ammoniacal and to contain organisms. Hence the walls of the bladder and the fluid in the interior were not modified by the heated paraffin in the first part of the experiment.

These experiments alone are sufficient to refute Liebig's view of organic molecules and decaying matter ; for in the first part of the experiment they were present in an unlimited amount, but so long as organisms were excluded no fermentation occurred. Their bearing also on the theory of spontaneous generation will be at once evident, and has indeed been already alluded to. I would only refer here to the experiments in which the urine was made *alkaline*, and in which therefore we had a natural alkaline urine full of organic molecules and in contact with the tissues of the bladder, and yet no organisms appeared in it so long as the dust of the air was excluded ; in other words, *the alkali had no influence in determining the re-arrangement of the numerous organic molecules which were present in the wall of the bladder and in the fluid in its interior, so as to form new living beings*. This is in exact correspondence with Dr. Roberts's results, and is a much more telling experiment.

Some very remarkable and convincing facts have been lately obtained by Dr. Paul Bert.[1]

On subjecting the 'unformed' ferments, such as ptyalin, pepsin, inversive ferment, myrosin, and emulsin to high degrees of pressure, he found that the properties of these ferments were not in any way impaired.

Thus to quote Experiment 467 :—

'21 Juillet 1874. Saliva humaine étendue d'eau et placée dans un matras étiré à la lampe, et soumis à 15 atm. d'un air suroxygéné.

'Le 30 Juillet je décomprime et soude l'extrémité du tube effilé.

'18 Janvier 1875. Cette saliva qui ne sent rien et paraît bien normale, neutre aux réactifs, transforme avec une grande énergie l'amidon cuit en glycose.'

It was proved by a former experiment (p. 201) that the amylolytic property of ptyalin was not altered by diminution of pressure, and the same is true when the pressure is increased:

[1] *La Pression barométrique.*

and, what is of great importance, other ferments of the same class, which are apt to lose their properties when kept, owing to the occurrence of putrefaction, retain these after being subjected to strong pressure if new causes of putrefaction are excluded. The explanation of this fact is simply that the bacteria and fungi are killed by the high pressure.

Bert also enquired whether these ferments could continue to act, in this compressed air, and he found that though they did continue to act the rapidity of their action was manifestly diminished. Thus Exp. 470 :—

'20 Janvier. Salive, amidon cru et eau. Bien mêlé et placé dans plusieurs tubes. On s'assure que le mélange ne contient pas de glycose.

'A—à la pression normale, bouché avec cornet de papier renversé.

'B—à 21 atm. d'air suroxygéné.

'Tous les deux sont mis à l'étuve, 30 degrés.

'25 Janvier. Essayé avec liqueur bleue :—

'A—7ce en réduisent 35 gouttes.

'B—7ce en réduisent 14 gouttes.'

This result was, however, not obtained unless the fluids were examined within a few days. At a later period, especially if diastase was employed, the contrary was found ; the fluid, subjected to compression, containing more sugar than the other. It was found that the explanation of this was that the diastase which had remained exposed to the air had become altered, owing to the growth of organisms in it, while organisms being unable to develop in that subjected to high pressure, the diastase had retained its properties and continued to act.

Paul Bert concludes : 'All the soluble false ferments with which we have experimented, diastase, ptyalin, pepsin, inversive ferment, myrosin, emulsin, have yielded the same result, and have retained their characteristic properties, after the prolonged action of oxygen, at a high pressure. Indeed, as this compressed oxygen destroys the germs of fungi, vibriones, &c., which sooner or later destroy these ferments when exposed to ordinary air, the latter remain unaltered for an apparently indefinite period of time.'

If we now compare these facts with those obtained by subjecting the 'true' ferments—those causing putrefaction, &c.—to

high pressures, we shall find a remarkable contrast; the latter class of ferments behave under high pressures like living beings, not like the unformed ferments just mentioned.

Bert says: 'The most striking fact which has been made out in these experiments is that, in air sufficiently compressed, putrefaction does not occur, no disagreeable odour manifests itself, and muscle, for instance, preserves its normal appearance except in colour; its microscopic structure is not markedly altered.'

Thus Exp. 404. '17 Mars. Viande en morceaux et eau; dans 2 petits matras effilés à la lampe. A—à la pression normale.

'B, B′—à 16 atmosphères d'une compression faite avec de l'air contenant 80 pour 100 d'oxygène.

'26 Mars. Décomprimé. A, pourri, infect. B n'a pas d'odeur et est neutre aux papiers réactifs.'

But this is not all; for when one restores the pressure to the normal, taking sufficient precautions to prevent the entrance of new organisms from without, putrefaction no longer occurs, and unboiled meat may be preserved at the normal pressure for an indefinite time after being subjected to high atmospheric pressures. The precautions required to prevent the entrance of organisms after the compression, and to ensure the complete destruction of those present, are detailed on p.184.

Exp. 407. '20 février. On met dans 15 tubes 15 morceaux de viande pesant chacun 1 gr. Ces tubes sont ensuite étirés à la lampe et soumis dans l'appareil en fer, à 15 atmosphères très-suroxygénés.

'3 Mars. On décomprime avec précaution et l'on ferme à la lampe les 15 tubes. L'analyse de 3 d'entre eux, faite aussitôt, donne de 70 à 80 pour 100 d'oxygène.

'13 Mars. 'On brise un des tubes sous le mercure: viande ambrée, pas d'odeur, réaction acide. On trouve 6·2 pour 100 d'acide carbonique et 77·8 d'oxygène.'

From a large number of experiments Bert finds that a pressure of twenty-one atmospheres is sufficient to kill the organisms which cause putrefaction. Similar facts were made out as to fermentations in blood, eggs, urine, milk, alcoholic fermentations, &c. A fact which he observed more especially in connection with blood and milk, is worth mentioning. He found that in the case of milk, as in the case of other substances,

putrefaction was arrested by compressed air. But if *tubes* were used neither coagulation nor rapid acidification were prevented. Was this because oxygen in tension was without action on the bacterium lactis? or was it that the coagulation of milk was not the work of these microscopic organisms, but rather of some agent which can resist oxygen, as we have seen to be the case with the 'unformed' ferments? On further investigation, however, he found that the result depended on the thickness of the layer of liquid. If it was thin the tendency to coagulation was destroyed.

Exp. 431. '10 Août. Lait bouilli; mis en couche de 2 à 3 millimètres d'épaisseur dans deux cristallisoirs neufs et bien lavés.

'A—à l'air libre, sous un verre qui arrête les poussières.

'B—à 25 atmosphères d'air suroxygéné.

'14 Août. Décomprimé.

'A est coagulé depuis le 11 et sent très-mauvais.

'B est liquide, ne sent aucune odeur et paraît normal.'

Paul Bert sums up his results with milk as follows: 'These experiments prove in a very conclusive manner that oxygen in a state of high tension prevents the coagulation of milk, that is to say, kills the vibrios which cause the lactic fermentation. As the action of these organisms is very rapid, it is necessary, in order to arrest it, to employ oxygen at very high pressures, and to have the fluid in a thin layer, so that the oxygen can saturate it quickly. In the case of putrefaction, which occurs much more slowly, these excessive precautions are not necessary; milk differing from blood in not consuming the oxygen as it penetrates the liquid, the gas has time to reach the bottom of the tubes and to kill all the putrefactive agents present. This fact explains how one can so easily, by means of compressed air, prevent milk from putrefying, and yet have so much difficulty in preventing its coagulation.'

It would be superfluous to pursue the proof of this matter further. The greater part of the preceding portion of this work has consisted of evidence which, taken together, can leave no doubt on the mind that putrefaction, like other fermentations, is caused by the growth of organisms in the putrefying material Pasteur's researches have led him further, and caused

him to adopt a theory of fermentation which has certainly many facts to support it, though I doubt if it can hold good in many instances. He thinks that when a substance putrefies two classes of microscopic organisms are at work, the first in point of time being chiefly engaged in abstracting the free oxygen from the material, and the second, which then appear, being unable to live in free oxygen, but nevertheless requiring oxygen for their growth, and obtaining it from the chemical combinations present. The result of this extraction of oxygen is the breaking up of these compounds—their putrefaction—and the rearrangement of their elements to form new compounds, which constitute the products of putrefaction. Whether Pasteur's theory be true or not, all the experimental results taken together, as well as the numerous facts known as to the power of antiseptics in arresting this class of fermentations, render it no longer doubtful that the particles which fall into organic materials and cause fermentations there, are the same as those which, falling on the same substances, give rise to the lower forms of organisms; in other words, are bacteria or their spores.

CHAPTER XII.

RELATION OF MICRO-ORGANISMS TO THE FLUIDS AND TISSUES OF THE LIVING BODY.

Proposed mode of enquiry—Does the aseptic method prevent putrefaction? Does it exclude organisms from wounds? Ranke's results: Klebs' objection: Ranke's reply: Demarquay: Fischer: Schüller: my own method—Results in aseptic wounds—Results in wounds treated otherwise—Koch's method of staining pus—Results in cases not treated aseptically—Examples of complete exclusion of organisms in aseptic cases—Examples of the entrance of micrococci in aseptic cases—Definition of micrococci—Distinctions between micrococci and bacteria. Are organisms present in the healthy living body?—'Bistournage.' Are organisms present in the body in states of disease?—Experiments with ammonia, phosphorus, &c.—The healthy blood and tissues can destroy organisms—Relation of organisms to abscesses. How do micrococci enter aseptic wounds? Carbolic lotion a sufficient germicide; Spray, its value—Stimson's experiments; Gauze dressing as a protection against entrance of organisms; Carbolic acid as a germicide in albuminous fluids; Relations of micrococci and bacteria to fluids containing carbolic acid. Conclusions.

ALL the experiments which have been referred to as yet relate to fluids and tissues removed from the body and preserved in flasks. It now remains, before quitting the subject, to enquire if our conclusions hold good for fluids and tissues retained in connection with the living body. An investigation of this sort has been demanded by some writers, as, for instance, by Mr. Holmes. At least that is what I take him to imply by the following passage[1] (I can see no other meaning in it): 'When we are told that, in order to practise antiseptic surgery, we must believe in the germ theory, then I cannot but say that belief is not a voluntary act; it must follow upon proof, and no convincing proof of the germ theory as applied to *living tissues and living phenomena* has, as far as I know, yet been offered.' Granting that I interpret Mr. Holmes' meaning aright, I venture to differ from him as to the necessity for such

[1] See MacCormac's *Antiseptic Surgery*, p. 51.