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.

facts, but nevertheless I think it well to introduce here some investigations which I have carried on with reference to this question.[1]

The mode in which I propose to ascertain whether the former conclusions apply to the living body or not is by the examination, in various ways and under varying circumstances, of fluids and tissues in the body.

We have before seen that the method which we have agreed to term the aseptic method of treatment is nothing more or less than a series of experiments on the germ theory of putrefaction—experiments made with the object of rendering atmospheric dust inert before it reaches the wounds. We shall attain the object of the present enquiry by ascertaining how far these experiments are successful. In discussing this question the following points suggest themselves :—

Does the aseptic method of treatment prevent putrefactive or other fermentations in the discharges or tissues of wounds?

If putrefaction is prevented are organisms also excluded?

If under any circumstances organisms do enter wounds so treated, what are the peculiarities of these bodies?

Are organisms present or do fermentations occur in fluids or tissues in the living body, which have never been exposed to the atmospheric dust?

If organisms are present, how is their occurrence to be explained?

If organisms enter wounds treated aseptically how do they get in?

1. First, then, does this method prevent putrefaction? Undoubtedly it does. Compare the course of an abscess, connected with diseased bone, opened and kept open without aseptic precautions, with that of one opened in accordance with strict aseptic principles. In the former case the pus rapidly undergoes fermentation, in all probability putrefaction; in the latter case the discharge does not undergo fermentation, and remains sweet and pure till healing is complete, however long that may be. I have at this present moment in my mind such a case. A patient, a young woman, came under Mr.

[1] For many details not mentioned here, see the *Transactions of the Pathological Society* for 1879.

Lister's care in August 1876, with spinal disease and psoas abscess. Subsequently a psoas abscess appeared on the other side, and later a lumbar abscess also. I dressed the case almost from the first myself, and though these dressings were changed at first daily and then ultimately weekly for nearly four years (for complete healing did not occur till June 1880, though there had been, for a long time, only minute sinuses furnishing almost no discharge), yet neither putrefaction nor any other fermentation ever occurred in the discharge from these wounds. Indeed, I may say, from long experience of Mr. Lister's practice and from long use of his method myself, that one who has had some experience in this method may now reckon with certainty on avoiding putrefaction or other fermentative change in discharges from any wound made in a situation where aseptic dressings can be applied, provided always that the treatment be strictly carried out by the method described at length at the beginning of this work.

2. Such being the facts with regard to the absence of putrefaction, is it equally the case that organisms are absent from the wounds? We saw a constant relation between the bacterium lactis and the lactic fermentation: can similar facts be found with regard to aseptic and septic wounds?

The first communication on this subject was made by Dr. Ranke,[1] of Halle, in 1874. He published a note of 300 examinations of the discharge from fifteen wounds treated aseptically, and following an aseptic course, in which he states that on only one occasion did he fail to find organisms. His method was simply to look at the discharge through a microscope, and it was not a particularly high power which he employed. The organisms which he says were present were for the most part micrococci in pairs, also streptococci; more rarely small or middle-sized bacteria. He did not carry his investigations farther, but on this evidence he rejects the germ theory as sufficiently explaining the etiology of septic diseases.

While by some these observations have been regarded as accurate and as confirming their previously formed views, by many they have been looked on as erroneous, either from having been made on cases in which the aseptic method had been

[1] *Chirurg. Centralblatt*, No. 13, 1874.

imperfectly carried out, or in themselves faulty. In answer to objections of the former nature urged by Professor Klebs[1] of Prague, Dr. Ranke[2] published another paper in July 1876, quoting cases to show that the treatment had been in reality properly carried out. He instances especially cases of hydrocele, treated by making a small incision into the sac with aseptic precautions and stitching it to the skin, where cure followed without any inflammation or constitutional disturbance, but where, nevertheless, organisms were present in the discharge. From those cases, as well as from the various published reports of the results of Professor Volkmann's practice, there seems no reason for doubting that the observations were made on wounds treated with all due precautions, and following an aseptic course similar to that which Mr. Lister himself would expect.

About the same time Demarquay[3] published the results of eight cases treated 'antiseptically,' in all of which organisms were found. The general course of the wounds so treated, as described by the author, and the fact that one of the eight cases died of pyæmia, show that whether the cases were treated *antiseptically* or no, they were not treated *aseptically*.

Two years later there appeared a paper by Dr. Fischer of Strasburg,[4] giving the result of investigations carried on in Professor Lücke's wards. He employed chemical tests, especially acetic acid and glycerine, as recommended by Von Recklinghausen, and he found organisms in all the cases examined. He, however, states that bacteria were not unfrequently present, his results differing in this respect from those of Dr. Ranke. Now, it so happens, I spent the summer of 1876 in Strasburg, and thus had frequent opportunities of seeing the 'aseptic practice' in that hospital, and I can only say that I was not surprised when I heard that bacteria had been found in the wounds.

The last paper on this subject was published by Dr. Schüller[5] in the spring of 1877. In his investigations attempts were made to cultivate organisms from wounds. He found that in many cases organisms were absent both from the discharge and the cultivating liquid, whilst in other cases they were present. He does not specify what the nature of these organisms was, and he is inclined to associate their presence in wounds with the occurrence of tension, &c. There are various objections to his results, but these I need not stay to discuss.

[1] *Archiv für Experimentelle Pathologie*, Bd. iii. p. 315.
[2] *Deutsche Zeitschrift für Chirurgie*, Bd. vii. p. 63.
[3] *Comptes-Rendus*, 1874.
[4] *Deutsche Zeitschrift für Chirurgie*, Bd. vi. p. 320.
[5] *Ibid.* Bd. vii.

As long ago as 1876 I began a series of investigations on this matter. My first observations were of the same nature as those made by Dr. Fischer; that is to say, I not only examined the discharges microscopically, but I also treated them with acetic acid and glycerine. These substances are recommended by Professor Recklinghausen for this purpose, the glycerine being supposed to dissolve the fat granules and the acetic acid to render the protoplasm invisible; thus, only nuclei and micro-organisms are left. On treating pus in this way I found that a large quantity of granular matter remained, and, though I very soon arrived at the conclusion that *bacteria—i.e.* rod-shaped organisms—are not present in the discharge from cases treated aseptically, I could not say whether among the granular matter seen there were or were not *micrococci*. This difficulty is the greater as there is more granular matter in aseptic wounds than in others.

I therefore soon commenced a series of cultivation experiments. The following was the principle on which I acted. On introducing a particular form of organism into a suitable pabulum with precautions against the entrance of others, this form of organism will grow there. This being the case, theoretically one would only require to inoculate some suitable pabulum with various discharges—on the one hand to get a development of organisms, on the other to find the fluid remain free from organisms, and unchanged. Various preliminary experiments, which I need not detail, established this. For the present investigation some suitable pabulum must be taken, sterilised, and inoculated under proper precautions with discharges from wounds. If we have a really pure pabulum, and the inoculation has been carried out in such a way as to prevent the entrance of any extraneous organisms, the inference, where development occurs, would naturally be that organisms have

been present in the fluid from which the inoculation was made. If, on the other hand, the same method has been employed, and no organisms develop, the inference would be that no organisms existed in the fluid.

I first used milk, but for various reasons I gave it up, and tried Pasteur's and Cohn's fluids, and, after reading Schüller's paper, Bergmann's; but I found these artificial solutions too insensitive to be of any value for my purpose. I then used vegetable infusions, more especially turnip, and ultimately infusion of cucumber, which last seems to be very sensitive. I also employed in many cases an infusion f meat.

The infusion having been prepared, is filtered, introduced by syphon into Mr. Lister's double-necked flasks, boiled for twenty minutes, kept for some days (at least two) in an incubator, and then decanted under a spray of carbolic acid into smaller purified flasks, which are likewise placed in an incubator for several days before being used. These flasks are covered with cotton wool caps purified by heat or carbolic acid, or they stand on a glass plate and are covered by a glass cap and a glass shade, as described before in the case of Mr. Lister's liqueur glasses.

For the purpose of inoculation, small capillary tubes, such as those used for vaccination, were employed. These possess the advantage over needles, in that, while they take up a larger quantity of the discharge, they protect it from the carbolic acid of the spray during the transit from the wound to the flask. The tubes are dropped into the flask containing the cucumber, and this is then placed in an incubator kept at the temperature of the human body. (See Fig. 72.)

The procedure may be shortly described as follows:—The outer portion of the dressing having been removed under the carbolic acid spray, a tube which has been previously purified in carbolic lotion is heated in the flame of a spirit lamp in the spray, so as to drive off all the carbolic lotion and to render it dry. This tube is now rapidly introduced into the drainage tube, and from thence immediately into the flask which is opened in the spray close to the wound. The flask is then placed in an incubator kept constantly at a temperature of 98° Fahr. In the case where flasks with cotton caps are used

it is well in performing an experiment to wet the margin of the cap with carbolic lotion before lifting it, so as to prevent any dust from falling from the cap into the fluid. This is a very important precaution.

Having ascertained that the method proposed was perfectly trustworthy, I proceeded to the investigation.

In performing the experiments I always inoculated two flasks, and often another was taken and the whole process gone through in the same place, with this difference, that the tube in the latter case, when heated, was put directly into the flask without touching the wound. These latter flasks remained, without exception, clear.

When development occurs in the flasks inoculated the fluid generally becomes muddy in 30 to 50 hours, but where the fluids remained clear I have kept them in the incubator for weeks, and then tested them by the addition of some substance containing bacteria.

FIG. 72.—FLASK CONTAINING CULTIVATING FLUID INOCULATED FROM A WOUND.

As a result, I find that in cases treated aseptically, where of course there was an unbroken skin to start with, one of two things may happen—either the fluid remains *perfectly clear*, without the development of organisms, showing that none were present in the wound; or the fluid *becomes turbid* from the presence in it of organisms of the form seen in Fig. 1, Plate I. In both cases the wound may follow an aseptic course; *i.e.* no local or constitutional disturbance results from the operation, and from the appearance of the wound one could not tell in many cases whether these organisms were present or absent.

From Fig. 1 it will be seen that these organisms are minute spherical bodies arranged in pairs; in triplets, in which case they form a triangle (a very important point in distinguishing them from other forms); in groups of four (positions which bacteria never take up); also in short chains and groups of larger or smaller size. In fact they belong to the group of the schizomycetes termed *micrococci*.

I have said that in many cases one could not tell from the

course of the wound whether these organisms are present or absent, but sometimes their presence can be suspected. Those who have worked long at aseptic surgery will have met with cases where when a dressing is left on for six or seven days, or when a deep dressing is left for some weeks, the discharge acquires a sour odour and the skin around the wound becomes somewhat excoriated. As the wound in other respects follows an aseptic course, Mr. Lister concluded that this was probably a chemical change taking place between the discharge and the materials in the gauze dressing. Knowing the peculiar property possessed by salicylic acid of preventing chemical fermentations, Mr. Lister uses it in such cases with the effect of diminishing or preventing this change.

In these cases I have always found micrococci.

If micrococci be grown in a small quantity (3 to 8 drachms) of cucumber fluid, after three days they seem to die; at any rate, they will not grow in any liquid. But yet if the fluid be kept for some weeks it will gradually become red, till it ultimately is of a dark vermilion tint. Thus chemical changes continue after the activity of the organism has ceased. May not something of the same kind occur in these cases? Chemical changes are primarily set agoing by these organisms, but continue of themselves, and thus salicylic acid acts by preventing these changes, as Mr. Lister supposed, though, according to this view, the organisms are necessary for their commencement.

If now we contrast these results with those obtained in wounds not treated strictly aseptically we find this marked difference, that in *none* of the latter were *organisms absent*, while in almost all *bacteria as well as micrococci* were present. It is to be observed that in many of the cases antiseptics were employed, both in the external dressings and injected into the wound, but no precautions were taken either to penetrate to all the recesses of the wound with the antiseptic so injected, or to prevent the access of organisms during and after the dressings.

I may mention that in four cases which were originally treated aseptically *bacteria* were found, but in all these their presence was indicated by disagreeable smell or by symptoms of local or constitutional disturbance. It is thus evident that bacteria as well as micrococci can flourish under an antiseptic

dressing. The explanation of their absence must therefore be that the circumstances which permit of the entrance of micrococci are not such as to allow the advent of bacteria.

It was thus satisfactorily established that there was a very marked difference between the discharges of aseptic wounds and of those not treated aseptically. From the former, organisms were generally *absent* till about the end of the case when the dressings were left on for several days. In the latter, organisms are *present*, even within the first twenty-four hours. Again, in the former, when organisms did appear they constantly belonged to the group of micrococci, in the latter rod-shaped organisms were frequently present as well, and generally in large quantities if there was any putridity in the wound.

It was just possible that an objection could be brought against these results to the effect that organisms might have been present in the discharge of aseptic wounds, but that they were unable to develop in the fluid used for cultivation. To obviate this objection as far as possible I used a variety of cultivating fluids and got the same results with all.

During the spring and summer of 1880 I renewed the study of this subject in a different manner. I adopted Koch's method of staining bacteria[1] and I employed it in all Mr. Lister's cases from the beginning of March till the end of June (four months), and my results confirm in every respect those which I had got by the method of cultivation.

I find that, in the first few days after an aseptic operation no organisms can be found in the discharge, and that, when they ultimately do appear, they are micrococci, not bacteria. On the other hand, after operations not performed aseptically organisms are generally present from the first, and as a rule these consist of bacteria as well as micrococci.

The principle of this method of staining is that various aniline dyes, more especially methyl violet, fuchsin and aniline brown, stain chiefly the nuclei of cells and bacteria; though these are generally the only bodies stained, yet in some cases, especially if the staining is excessive, other albuminous granular matter may also become coloured. However, even where such is the case

[1] See Cohn's *Beiträge zur Biologie der Pflanzen.*

the organisms can, as a rule, be easily recognised by their form and arrangement. The pus or other fluid to be examined is spread in a very thin layer on a cover glass or slide, and left to itself to dry or dried over a spirit lamp. In the case of albuminous fluids it is well to do nothing more for at least twenty-four hours. These cover glasses may be kept for months and then used, for no organisms can grow on the dried materials.

In order to stain the specimens a few drops of a saturated solution of methyl violet or of fuchsin in alcohol are added to distilled water till a sufficient depth of colour is obtained. This can only be determined by experience, but it is well to stop before any precipitation can be detected. (Dr. Ogston recommends a watery solution of methyl violet of the strength of half a grain to the ounce.) A drop of this solution is allowed to flow over the cover glass, being retained in contact with the material to be stained for about one minute. It is then washed off with distilled water and the cover glass again dried as before. When quite dry it is mounted in Canada balsam.

When aniline brown is used a concentrated solution in glycerine is prepared. This is filtered, and one part of the filtrate is added to an equal quantity of distilled water and an equal quantity of pure glycerine. This mixture is now filtered and is then ready for use. I find that this fluid, while it does excellently for staining organisms in such fluids as cucumber, turnip infusions, &c., does not stain them well in pus. I find it best in the latter case to place a drop of the staining fluid on a slide, then lay on the cover glass, the material to be stained being of course lowermost. Leave this for twenty-four hours and then suck out the staining fluid with filter paper, introducing in its stead pure glycerine. This is a difficult process, and the specimens are frequently not quite clean. However, if one examines the layer attached to the cover glass, one sees what was in the material; the fragments which are floating free may consist of all sorts of débris. The specimen is then surrounded with cement.

The results of these methods of staining are very beautiful. If the staining is not too intense, only the nuclei of the pus-cells and any organisms which are present are stained, and the latter can be recognised with the greatest readiness with a sufficiently high power.

So much for the method. Plates I. to IV. illustrate the results.

Let us take first some specimens from wounds which have not been treated aseptically. Here it will be seen that there are always organisms, and that these generally consist both of bacteria and micrococci, though sometimes of one or other alone. Look at any wound not treated aseptically, which has not united by first intention, and which has been somewhat recently made, and you will get this result.

Case 1.—Fig. 2 is a specimen of the discharge taken from a compound dislocation of the thumb a few days after the accident. The wound had not been treated aseptically, and it had a very foul smell. (The patient, by the way, died of tetanus.) Here a great variety of organisms will be found—bacteria, bacilli of various kinds, and micrococci.

Case 2.—Fig. 3 is a specimen of discharge taken from a wound of the scrotum in which a small slough was lying. The wound was syringed out daily with carbolic lotion 1–40, and dressed with boracic ointment. The discharge had a very foul smell. Here there are multitudes of minute bacteria, bacilli, and micrococci.

Case 3.—Fig. 4 was taken from a case in the out-patient room, not treated aseptically. There was not much discharge and no putrid odour; rather a slightly rancid smell. Here well-marked bacilli can be seen. Discharge taken on two occasions presented the same appearance.

Case 4.—Fig. 5 was taken from a case of amputation of the thigh which had been done two days previously, and had been treated by irrigation, though I must say, for the credit of irrigation, not very efficiently. Here there was a slight smell. Bacilli are present.

Case 5.—Fig. 6 was taken from a case of excision of the hip-joint where numerous sinuses existed previous to the operation, and where, therefore, there was no hope of eradicating putrefaction. I dressed this case myself, washing out all the sinuses daily with 1–40 carbolic acid lotion, and applying boracic or salicylic acid ointment, and outside this boracic lint. The specimen figured was taken more than four months after the operation, and contains numerous bacteria; this, observe, although the wound had been treated assiduously for months with antiseptics, but not aseptically.

At the end of March some pieces of dead bone were felt, and these were removed on April 1st. The wound and sinuses were thoroughly washed out with chloride of zinc and dressed as before.