THE INFLUENCE OF DIFFERENT KINDS OF SOIL
ON THE
COMMA AND TYPHOID ORGANISMS.

BY

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COBB RESEARCH SCHOLAR, 1893.

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Received May 8th—Read May 22nd, 1894.

THE influence of external conditions upon pathogenic organisms is a subject that has naturally engaged the attention of bacteriologists for a considerable time ; and the important bearing which researches in this direction have upon the epidemiology of disease will prove a sufficient stimulus for prolonged investigation in the future. Of such enquiries we cannot have too many, as upon the facts which they succeed in demonstrating the foundations of hygiene will ultimately rest.

It was the importance more than the novelty of the subject that led me to undertake this investigation, the results of which I now have the honour to lay before you. The work was undertaken with a view to endeavour to answer the following special question :

Has the soil in itself any action favourable or injurious to the life of the comma bacillus of cholera Asiatica, and the bacillus of typhoid fever? or does the length of life of these organisms in the soil simply depend upon the amount of moisture that may be present?

The action of the saprophytic bacteria present in the soil was left out of consideration altogether. For the purposes of this research *sterilised soils* alone were used.

The experiments were carried out with four different kinds of soil, namely, white crystal sand, yellow sand, garden earth, and peat.

FIRST SERIES OF EXPERIMENTS.

To find the length of time that the soils should be exposed to steam in Koch's apparatus to render them sterile.

Samples of the soils to be used in this research were placed in glass vessels provided with cotton plugs, and sterilised. After sterilisation gelatine-plate cultures, also beef-broth inoculations, were made.

It was found that a small quantity of soil can be sterilised by steam in Koch's apparatus, if exposed for one hour on three successive days to the temperature of boiling water.

SECOND SERIES OF EXPERIMENTS.

To find how long comma bacilli will live in a natural dry soil.

Three kinds of soil, namely, white crystal sand, yellow sand, and garden earth were passed through a sieve to remove stones, &c.

A measured quantity of each kind of soil was put into sterilised test-tubes and sterilised.

Mode of procedure.—The cholera bacilli were suspended in $\frac{3}{4}$ per cent. salt solution, or in distilled water, and for this purpose agar-agar cultures were

used, and the growth mixed with the sterilised salt solution or distilled water so as to form a faint milky emulsion; a measured quantity of this emulsion was added to each of the soils, not exceeding 1 c.c.

FIG. 1.

The tubes were either kept at room temperature or at 22° C. From time to time gelatine-plate cultures were made from the soils and, after incubation, examined for the presence of bacilli.

The *peptone water medium* as used by Koch was found of great service in the examination of the soil for comma bacilli. This peptone water was made as follows:—1 per cent. peptone, and 1 per cent. sodic chloride, were dissolved in 100 parts of water and the fluid rendered strongly alkaline.

The comma bacilli are more readily detected by this medium than by the ordinary plate methods. I found in the course of my experiments that the comma bacilli were often detected by means of peptone water cultivations when gelatine plates made from the same soil gave negative results. The peptone water, after being inoculated with the soil, was kept at blood heat. The comma bacilli multiply very rapidly in this medium, and rise to the surface: the result is that if one examines the peptone tubes after eight to twelve hours, one finds the comma bacilli in large numbers in the upper portion of the fluid; they are then readily detected by the microscope, and if plates be made from this peptone water there is no difficulty in obtaining typical colonies of the comma bacillus. The following method gives the best results, namely, first, to add the soil to peptone water, and then after twelve hours to make plates from the peptone water, instead of making plates directly from the soil.

By means of the methods described above, the results to be detailed were obtained. This series of experiments

was made with tubes plugged with cotton-wool (Fig. 1) and kept at 22° C. Consequently the soils lost their moisture.

In the white crystal sand, the yellowsand, and the garden earth, the comma bacilli were alive on the third day.

In the above three soils the comma bacilli were dead on the fifth day. Therefore the comma bacilli lived in these soils from three to five days.

The soils in the above experiments were air-dry, and only contained traces of moisture when the cholera emulsion was added to them.

THIRD SERIES OF EXPERIMENTS.

To find how long comma bacilli will live in a soil that has been rendered moist, without, however, containing any excess of moisture.

A measured quantity of white crystal sand, yellow sand, and garden earth was placed in sterilised test-tubes; these were made moist, and sterilised. Control experiments were then made to make sure that the soils were sterile before being used. To each of the three kinds of soil was added 1 c.c. of the watery emulsion of comma bacilli.

These experiments were made with tubes plugged with cotton wool and kept at 22° C.; consequently the soils gradually lost the moisture they contained.

The results obtained with gelatine plates were as follows:

In moist white crystal sand comma bacilli were found up to the seventh day. In moist yellow sand and in moist garden earth the comma bacilli were alive on the thirty-third day.

From these experiments it will be seen that the comma bacilli *lived longer in the moist soils* than was the case in the air-dry soils.

FOURTH SERIES OF EXPERIMENTS.

To find the length of time comma bacilli will live in a soil when any excess of moisture in the soil is allowed to escape through the soil, but where loss of moisture does not take place from the surface of the soil.

Instead of using ordinary test-tubes a special form of tube was obtained, as shown in Fig. 2. At the constriction A a layer of cotton-wool was placed, to form a support for the soil above it and also to allow any excess of moisture in the soil to pass into the lower part of the tube. The tubes were plugged with cotton-wool and sterilised.

In three such sterilised tubes a measured quantity of soil was placed, and well sterilised.

The soils used were white crystal sand, yellow sand, and garden earth.

About 3 c.c. of an emulsion of comma bacilli was added to each of the soils; the tubes were provided with tight, well-fitting india-rubber caps to prevent loss of moisture, and kept at a temperature of 18° to 20° C. Any excess of moisture was able to pass through the layer of cotton-wool under the soil into the lower part of the tube.

Gelatine-plate cultures and peptone water inoculations of the soils were made from time to time to see if the bacilli were alive.

The results were as follows:

In white crystal sand the comma bacilli were found alive on the twenty-eighth day.

In yellow sand and in garden earth the comma bacilli were found alive on the sixty-eighth day.

FIG. 2.

FIFTH SERIES OF EXPERIMENTS.

To find how long the comma bacilli will live in dried soils.

White crystal sand, yellow sand, garden earth, and peat having been passed through a fine sieve, were then dried in porcelain dishes over the Bunsen flame, and a measured quantity of each was placed in sterilised test-tubes and the whole sterilised. To each of the four kinds of soil was added 1 c.c. of an emulsion of comma bacilli. These experiments were made with tubes plugged with cotton-wool and kept at blood heat, and under these conditions the soils quickly lost any added moisture.

The results obtained with gelatine-plate cultures were as follows :

In none of these dried soils were comma bacilli found at the end of twenty-four hours.

In one or two experiments they were found alive in the white crystal sand, yellow sand, and garden earth after twenty-four hours, but not after forty-eight hours.

The comma bacilli did not therefore live in the dried soils longer than one to two days. *The only exception was peat, in which the comma bacilli were invariably dead within twenty-four hours.* The results obtained with a peat soil will be specially referred to later on.

If we compare the above series of experiments we get the following results :

On a dried soil, the comma bacilli lived one day.

On an air-dry soil, the comma bacilli lived three to five days.

On a moist soil in which a gradual loss of moisture took place, the bacilli lived seven to thirty-three days.

On a moist soil in which little or no loss of moisture took place, the bacilli lived twenty-eight to sixty-eight days.

On a moist soil, in which, however, loss of moisture was taking place gradually, the bacilli were not alive in a

control experiment made on the fifty-eighth day ; whilst on a moist soil losing little or no moisture, it will be seen that the bacilli were alive on the sixty-eighth day.

These experiments point to the close connection existing between the amount of moisture present in the soil and the length of life of the bacilli.

With a view of controlling these results a series of parallel experiments were made with white crystal sand. The results obtained were as follows :

In the white crystal sand *which had lost its moisture*, the comma bacilli were alive on the third day, but were dead on the eighth day.

In the white crystal sand *which was kept moist*, the comma bacilli were still alive on the forty-seventh day.

Therefore the comma bacilli lived longer in the soil that was kept moist.

A series of experiments was made to find the amount of moisture that is present in each of the soils used in these experiments.

It was found that—

The white crystal sand contained 0·1 per cent. of moisture.

The yellow sand ,, 1·18 ,, ,,

The garden earth ,, 2·2 ,, ,,

The peat ,, 25·34 ,, ,,

Each of these soils before being weighed was passed through a fine sieve to remove stones, &c., and it is in this fine condition that the soils have been used in these experiments.

SIXTH SERIES OF EXPERIMENTS.

To find the rate at which the soils lose their moisture by evaporation after the addition of an emulsion of comma bacilli, and kept at 18° to 20° C.

White crystal sand, yellow sand, garden earth, and peat were used in these experiments.

The results were as follows :

	per cent.		
White crystal sand	lost 0·304	of its moisture	per day.
Yellow sand	„ 0·292	„	„
Garden earth	„ 0·302	„	„
Peat	„ 0·297	„	„

These experiments show that when these four soils are placed under the same conditions of temperature they lose their moisture at a certain rate, *which is about the same in all the soils*. But in nature, owing to local and seasonal conditions, the rate of loss would not be the same from all soils. Thus good drainage will carry off the moisture more quickly ; and one soil may drain more quickly and more thoroughly than another, thus parting with its moisture more quickly.

The amount of natural moisture in each of these four kinds of soil varied greatly, and that soil which contains the least moisture (or which can part with its moisture in the shortest time by drainage or evaporation) is the one in which the bacilli might be expected to live the shortest time.

Under equal conditions of temperature, the *loss* of moisture by evaporation was about the same in white crystal sand, yellow sand, garden earth, and peat. The *amount* of natural moisture present in these soils, however, was different. The white crystal sand contained the least, garden earth and peat the most moisture. Sand being a surface soil will lose its moisture most quickly by evaporation and drainage, and in it under natural conditions the life of the organisms is therefore likely to be the shortest.

SEVENTH SERIES OF EXPERIMENTS.

To find the relation between the amount of moisture in the soil and the life of the comma bacillus.

Rationale of the process.—Having obtained the weight of a sterilised test-tube with its cotton-wool plug, a given

quantity of soil was weighed out and placed in this tube, and then sterilised; the emulsion of cholera bacilli was added, and the tube weighed so as to obtain the weight of the cholera emulsion added. The soil was kept at a temperature of 18° to 20° C., and weighed daily; the loss of weight represented the loss of moisture. Gelatine-plate or peptone-water cultures of the soil were made from time to time, the weight being also noted, until the bacilli were found to be dead. We thus obtained the time when the bacilli were last found alive, and also the amount of moisture that had escaped from the soil, and consequently the amount of moisture left in the soil and the point of dryness of the soil below which the bacilli cease to live.

This series of experiments was performed with white crystal sand and yellow sand, in tubes plugged with cotton wool and kept at 18° to 20° C. so that the soils gradually lost their moisture; as well as in tubes closed with a tight, well-fitting india-rubber cap (and kept at 18° to 20° C.), by means of which the loss of moisture was very considerably delayed.

The results obtained with white crystal sand were as follows:

In the white crystal sand *where the loss of moisture went on quickly* comma bacilli were alive on the twenty-seventh day, but were dead on the thirtieth day: and it was found that on the twenty-seventh day, when the bacilli were alive, the sand contained 1.572 per cent. of moisture; while on the thirtieth day, when the bacilli were dead, the soil only contained 0.66 per cent. of moisture.

In the white crystal sand *where the loss of moisture was almost prevented* comma bacilli lived much longer, and were alive on the 174th day, and the sand contained 7.1 per cent. of moisture.

The results obtained with yellow sand were as follows:

In the yellow sand *where the loss of moisture was rapid* the comma bacilli were dead on the twelfth day, when the soil contained 1.44 per cent. of moisture. And in the yellow sand *where the loss of moisture was almost pre-*

vented the bacilli were alive on the thirty-sixth day, when the soil contained 8·659 per cent. of moisture.

These experiments illustrate the close relation existing between the amount of moisture present in the soil and the length of life of the micro-organisms. For example, in white crystal sand containing 7·1 per cent. of moisture the bacilli were found alive up to the 174th day; in white crystal sand containing 1·57 per cent. of moisture the bacilli were found alive up to the twenty-seventh day, but on the thirtieth day, when the amount of moisture had fallen to ·66 per cent., the bacilli were dead.

EIGHTH SERIES OF EXPERIMENTS.

To find the length of life of the comma bacilli in sterilised ·75 per cent. salt solution.

The comma bacilli were obtained from pure cultures on nutrient agar-agar.

10 c.c. of ·75 per cent. salt solution was placed in a sterilised test-tube and sterilised.

Into this sterilised salt solution was introduced some of the pure culture of comma bacilli, and kept at 18° C.

When the comma bacilli had been in this salt solution for 159 days, a tube of peptone water was inoculated from the salt solution and placed in the 37° C. incubator for twenty-four hours. On examination the peptone water was cloudy, and had a distinct surface pellicle. A microscopical preparation of this pellicle revealed comma bacilli.

The comma bacilli had remained alive in the salt solution for 159 days; beyond which time the experiments were not carried.

NINTH SERIES OF EXPERIMENTS.

To find the length of life of the comma bacilli in sterilised acid urine.

Into sterilised test-tubes about 20 c.c. of fresh acid urine was placed, and sterilised in the usual way. The tubes were then inoculated with a pure culture of the comma bacilli. Some of these tubes were kept at blood heat (37° C.) ; others at 22° C.

Gelatine-plate cultivations and peptone-water inoculations were made from time to time with the urines, and examined microscopically.

The results obtained were as follows :

The comma bacilli lived in urine at blood heat for 14 days.

“ “ “ “ 22° C. “ 29 “

TYPHOID.

TENTH SERIES OF EXPERIMENTS, WITH THE BACILLUS OF TYPHOID FEVER.

To find how long typhoid bacilli will live in a natural dry soil.

Three kinds of soil, namely, white crystal sand, yellow sand, and garden earth were passed through a sieve to remove stones, &c.

Into sterilised Erlenmeyer flasks and test-tubes a measured quantity of each kind of soil was put, and sterilised.

Inoculations from control flasks proved that the soil contained no living organisms.

An agar-agar culture of typhoid bacillus was taken and a faint milky emulsion made in sterilised 75 per cent. salt solution.

To each of the soils was added 1 c.c. of an emulsion of typhoid bacilli.

These experiments were made with flasks and tubes plugged with cotton wool (Fig. 3) and kept at 22° C.; consequently the soils gradually lost their moisture.

FIG. 3.

The results obtained with gelatine-plate cultures were as follows :

In the white crystal sand the typhoid bacilli died between the ninth and the eleventh days.

In the yellow sand they died between the eighteenth and twentieth days.

In the garden earth they died between the fourteenth and the eighteenth days.

TENTH SERIES OF EXPERIMENTS (*continued*).

To find the length of time typhoid bacilli will live in peat.

The peat having been passed through a sieve, a measured quantity was placed in sterilised test-tubes, and the whole sterilised. To each was added 5 c.c. of an emulsion of typhoid bacilli. The tubes were plugged with cotton wool and kept at 32° C.

The results obtained with gelatine-plate cultures were as follows :

The typhoid bacilli were dead at the end of twenty-four hours.

Typhoid bacilli were found alive at the end of one hour, but not at the end of twenty-three hours.

In the case of the comma bacilli it was found that they died on a peat soil *within twenty-four hours*.

The typhoid organisms were also dead within twenty-four hours. These results were quite independent of the amount of moisture present in the peat. The peat had therefore a marked destructive action on the micro-organisms even when an abundance of moisture was

present. Peat was *the only one* of the soils experimented with which proved distinctly inimical to the life of these microbes, and its action was rapid and energetic.

ELEVENTH SERIES OF EXPERIMENTS.

To find the length of time that typhoid bacilli will live in a soil that has been rendered moist.

Into sterilised test-tubes was put a measured quantity of white crystal sand, yellow sand, and garden earth; these were rendered moist, and then sterilised.

To each of the three kinds of soil 1 c.c. of an emulsion of typhoid bacilli was added.

The experiments were made with tubes plugged with cotton wool, and kept at 22° C.; and the soils gradually lost their moisture.

The results obtained with gelatine plates were as follows:

In the moist white crystal sand typhoid bacilli were alive on the twenty-third day, but were dead on the twenty-seventh day.

In the moist yellow sand and moist garden earth the typhoid bacilli were alive on the forty-second day.

TWELFTH SERIES OF EXPERIMENTS.

To find the length of time typhoid bacilli will live in dried soils.

White crystal sand, yellow sand, and garden earth having been passed through a fine sieve and well dried, a measured quantity of each was put into sterilised test-tubes, and the whole sterilised.

To each of the three kinds of soil was added 1 c.c. of an emulsion of typhoid bacilli.

These experiments were made with tubes plugged with cotton wool and kept at blood-heat (37° C.); and the added moisture gradually diminished.

The results obtained with gelatine plates were as follows:

The typhoid bacilli were found to be alive in all three kinds of soil on the seventh day, but were dead in all three on the ninth day.

To sum up—On a *dry* soil where evaporation was allowed to take place the following results were obtained :

In white crystal sand the bacilli were found up to the ninth day, in yellow sand up to the eighteenth day, and in garden earth up to the fourteenth day.

On the *moist* soils, on the other hand, the following were the results :

In moist white crystal sand the typhoid bacilli were alive on the twenty-third day ; in yellow sand and on garden earth on the forty-second day.

On soils which had been deprived of their moisture the bacilli were only found up to the seventh day.

THIRTEENTH SERIES OF EXPERIMENTS, MADE TO TEST THE FILTERING CAPACITIES OF THE SOILS FOR BACTERIA.

FIG. 4.

Glass tubes were used, as shown in Fig. 4. At the constriction B was placed a layer of wire gauze to support the soil, and on this rested a layer of soil six inches in depth. The end of the tube c was plugged with cotton-wool. The end A was provided with a perforated rubber stopper ; through the perforation was passed a glass tube having its external end plugged with cotton-wool.

A layer of six inches of the soil to be tested was introduced. The soils used were white crystal sand, yellow sand, and garden earth. The open ends of the tubes were plugged, and the tubes of soil well sterilised by steam.

An emulsion of typhoid bacilli was made by taking

an agar-agar culture of the bacilli, and mixing the growth with 75 per cent. salt solution till a faint milky emulsion was produced.

This emulsion of typhoid bacilli was taken up in sterilised pipettes and placed on the surface of the soils. Gelatine-plate cultures were made of the fluid that passed through the soils (the filtrates), and these plates were examined for typhoid bacilli, or for counting the colonies.

1st. 4 c.c. of this typhoid emulsion was placed on the surface of the *white crystal sand*. Some of it passed through the soil, and a gelatine-plate culture was made of the filtrate, and colonies of typhoid bacilli developed in the plate. The typhoid bacilli were therefore carried through six inches of the white crystal sand.

2nd. 2 c.c. of an emulsion of typhoid bacilli was poured on the surface of the *yellow sand*, and four days later some sterilised distilled water was allowed to fall on the sand to see if it would carry down any typhoid bacilli through the soil; some of this water passed through the six inches of yellow sand, and a gelatine-plate culture was made of this filtrate; on incubation this plate developed typical colonies of the typhoid bacilli.

3rd. 2 c.c. of the emulsion of typhoid bacilli was placed on the *garden earth*, and four days later some sterilised distilled water was allowed to fall on the surface of the soil, to see if it would carry down any typhoid bacilli; some of this water passed through the soil, and a gelatine-plate culture was made of this filtrate on which developed colonies of the typhoid bacilli. The typhoid bacilli were thus carried through six inches of garden soil.

Resumé.—Typhoid bacilli passed through six inches of white crystal sand, and were found in the filtrate on the fifth day.

Typhoid bacilli passed through six inches of yellow sand, and six inches of garden earth, and were found in the filtrates on the fourth day.

FIG. 5.

FOURTEENTH SERIES OF EXPERIMENTS.

Can the bacilli be carried by a current of water through a layer of sand two feet six inches in depth?

The sand on the surface of a filter-bed is 2 feet 6 inches in depth.

A glass tube was used, as shown in Fig. 5. At the constriction B was placed a layer of wire gauze to support the soil, and on this gauze rested a layer of white crystal sand 2 feet 6 inches in depth. The end of the tube at A was provided with a perforated rubber stopper, through which passed a glass tube having its external end plugged with cotton wool. The lower end of the tube was pointed, and passed through a rubber stopper into a glass flask; this rubber stopper should have a perforation through it containing a small glass tube plugged with cotton wool, so as to allow air to escape from the flask while its place is being taken up by water falling into the flask from the filtration tube.

The white crystal sand for this experiment was sterilised by heating it in a vessel over the Bunsen flame.

The filtration tube was sterilised with corrosive sublimate solution, and then washed well with sterilised distilled water.

The flask was freed from micro-organisms by dry heat in the sterilising oven.

White crystal sand, to the depth of 2 feet 6 inches, was placed in the filtering tube. This

sand was quite dry, all moisture having being driven off while sterilising it over the Bunsen flame, so that to resemble a working filter it was made moist again with water. A known quantity of sterilised distilled water was poured on the surface of the sand till it began to fall into the flask; the tube was left for twenty-four hours, so that all excess of water might drain from the soil; the flask was then removed, and a freshly sterilised one took its place. The next step was to see if water containing comma bacilli would pass through this 2 feet 6 inches of white crystal sand; for this purpose an emulsion of comma bacilli was made with 250 c.c. of sterilised distilled water. The whole of this emulsion was by degrees poured on the surface of the sand, the time taken being one hour and a quarter for the emulsion to pass through the sand into the flask below. Gelatine-plate cultures and peptone-water inoculations were made of the filtrate, and comma bacilli were found.

The comma bacilli were therefore carried by the water through 2 feet 6 inches of white crystal sand. It was found that the dry sand retained 60 c.c. of the sterilised distilled water that was poured on it to make it moist, and that after pouring 250 c.c. of the emulsion of cholera bacilli on to the sand 250 c.c. of fluid passed into the flask, so that at the end of this experiment the sand still retained 60 c.c. of moisture.

A gelatine-plate culture was made of the cholera emulsion before it was poured on to the sand; $\frac{2}{100}$ c.c. of the emulsion was used to make this plate. A gelatine-plate culture was also made of the fluid that had passed through the sand, $\frac{6}{100}$ c.c. of the filtrate being used for this purpose. These two plates were made the same day, and on examination the plate made from the filtrate did not contain nearly so many colonies of the comma bacilli as the plate made from the emulsion, although twice the quantity of filtrate was used to make the plate. Therefore, although some comma bacilli passed

through 2 feet 6 inches of sand, many have remained in the soil.

The next point was to determine if the comma bacilli remaining in the sand would be carried down into the flask by pouring some water on to the surface of the sand.

Twenty-four hours after the emulsion of comma bacilli had been placed on the soil, 250 c.c. of sterilised distilled water was poured by degrees on to the surface of the sand; it passed through the sand in fifteen minutes into a freshly-sterilised flask; this may be called the "second filtrate," and measured 250 c.c. A peptone-water inoculation on examination showed the presence of comma bacilli.

Although the sand therefore held back many comma bacilli during the first filtration of water contaminated with the bacilli, a further supply of fresh water on the surface of the sand carried down some of those bacilli that had been held back, and the second filtrate also contained comma bacilli.

Four days after the emulsion of comma bacilli had been placed on the soil, another 250 c.c. of sterilised distilled water was poured on the surface of the sand, to see if any more comma bacilli would be carried down into another freshly sterilised flask. The water was poured on the sand quickly, and at the end of nine minutes all the water had entered the surface of the sand, and was beginning to fall into the flask. This is the "third filtrate," and measured 250 c.c. The water fell from the soil into the flask in drops, not in a stream. A peptone-water inoculation on examination showed the presence of comma bacilli.

From the above it is seen that even a second supply of water on the surface of the filter carried down more comma bacilli, and that the third filtrate contained bacilli.

On the eighth day after the emulsion of comma bacilli had been placed on the soil, another 250 c.c. of sterilised distilled water was poured on the surface of the sand, to see if any more comma bacilli would be carried down from the soil. The fluid passed through the sand, and this

fourth filtrate measured 245 c.c. A peptone-water inoculation on examination showed that there were *no* comma bacilli in this fourth filtrate.

The filtering capacity of filter beds depends upon the upper layer of sand. Fresh sand is not an efficient filter for micro-organisms ; it is only when the suspended matter present in the water sinks and forms a film on the surface of the sand, that the bacteria present in the water are held back to any extent. The bacteria also establish themselves in the pores of the sand, and in this way also render the sand more impervious to the passage of bacteria. It will be seen, in the above laboratory experiment that, using the same sand filter two and a half feet thick, the first, second and third filtrates contained comma bacilli, but the fourth filtrate contained none.

FIFTEENTH SERIES OF EXPERIMENTS.

FIG. 6.

To find the number of bacilli that are held back during filtration of cholera-contaminated water through six inches of white crystal sand, the sand being dry as it would be in a new filter.

White crystal sand was placed in a filtration tube to the depth of six inches ; the tube was plugged (Fig. 6), and the whole well sterilised by steam.

With a sterilised pipette, 20 c.c. of an emulsion of comma bacilli was placed on the surface of the sand ; at the end of two minutes some of the emulsion had passed through the sand, and at the end of five minutes filtration appeared to be completed ; the amount of filtrate measured 10 c.c. It should be noted that this sand is in

its natural condition ; no water had been poured on it, so its pores were not saturated with moisture. It resembled a newly-made filter, or a surface of sand after dry weather.

The result with a filter of dry sand six inches thick was that 99·6 per cent. of the comma bacilli were held back.

SIXTEENTH SERIES OF EXPERIMENTS.

To find the number of bacilli that are retained in a filter of white crystal sand six inches in thickness when the sand is in a moist condition.

White crystal sand was placed in a filtration tube to the depth of six inches, the plugs were then placed in the two ends of the tube, and the whole sterilised in Koch's steam apparatus.

Sterilised water was poured on the sand till the whole was moistened, and the water passed through the sand in the bend of the tube. The soil was allowed to drain till the next day, when this water was decanted from the bend of the tube, and the bend was sterilised by heating it in the Bunsen flame in case air-borne germs had entered it during decanting.

With a sterilised pipette $13\frac{1}{2}$ c.c. of an emulsion of comma bacilli was placed on the sand ; in one minute and fifteen seconds some of the emulsion or filtrate had passed into the bend of the tube. The amount of filtrate measured exactly $13\frac{1}{2}$ c.c.

A gelatine-plate culture was made of some of the cholera emulsion before it was poured on the sand, to ascertain the number of bacteria it contained before being filtered ; $\frac{3}{100}$ ths of a c.c. of the emulsion was used to make the plate. At the same time a gelatine-plate culture was also made of some of the filtrate, to ascertain the number of colonies that would be produced after filtration ; $\frac{3}{100}$ ths of a c.c. of the filtrate was used to make this plate. Colonies of comma bacilli developed in these plates, and they were counted as before.

The plate made with $\frac{3}{100}$ ths c.c. of the emulsion contained 1425 colonies, so that 1 c.c. of it contained 47,500 micro-organisms before being filtered.

The plate made with $\frac{3}{100}$ ths c.c. of the filtrate contained only 48 colonies, so that 1 c.c. of it contained 1600 bacteria after filtration.

As each c.c. of the cholera emulsion is filtered through six inches of sand, 45,900 comma bacilli (47,500 less 1600) are held back by the sand; that is to say, 96.6 per cent. of the comma bacilli are held back by a filter of moist white crystal sand six inches in thickness.

The results of these experiments with a moist sand filter, then, showed that 96.6 per cent. of the comma bacilli were held back.

SEVENTEENTH SERIES OF EXPERIMENTS.

To determine the number of bacilli that are retained by a filter of yellow sand six inches in thickness when the sand is in a moist condition.

It was found that the moist yellow sand held back 99.9 per cent. of the comma bacilli, as compared with 96.6 per cent. held back by the moist white crystal sand.

EIGHTEENTH SERIES OF EXPERIMENTS.

To determine the number of bacilli that are retained in a filter of garden earth six inches thick when the soil is in a moist condition.

It was found that moist garden earth held back 89 per cent. of the comma bacilli.

NINETEENTH SERIES OF EXPERIMENTS.

To find the number of bacilli that are retained by a filter of peat six inches in thickness.

The result was that no comma bacilli were found to pass through a filter of moist peat six inches in thickness, or I would rather say no *living* bacilli passed through the peat filter. In my previous experiments I found that the peat quickly killed the comma and the typhoid organisms.

The following are the results of the filtering experiments:

A filter, six inches thick, composed of—

			Per cent.	
White crystal sand (dry)	holds back	99·6	of the comma	
				bacilli.
„	„ (moist)	„	96·6	„
Yellow sand	„	„	99·9	„
Garden earth	„	„	89	„
Peat	„	„	100	„

RESULTS.

I have now given the essential points in my investigation, and it only remains to sum up shortly its results. I found that white crystal sand, yellow sand, and garden earth have *no* marked favourable or injurious action on the life of the comma bacillus of cholera Asiatica, nor on the bacillus of typhoid fever. The all-important factor determining their length of life in these soils is *moisture*. We saw how the comma bacilli quickly died when planted on a given *dried* soil, and how their life on the same soil was prolonged by simply adding water to the soil in question. These results apply also to the typhoid organisms. *Peat*, on the contrary, proved very deadly to both the comma bacilli and the typhoid bacilli. Peat was the only one of the four soils which, *per se*, and independently of the amount of moisture present, exercised a distinctly inimical action on the comma and typhoid bacilli.

In sterilised salt solution the comma bacilli were alive on the 174th day. This experiment I was not able to carry further ; but it seems proved by Koch and others that in fluid media at any rate the presence of sodic chloride greatly favours the life of the comma bacilli.

As regards urine, the bacilli lived longer at 22° C. than at blood heat.

All these experiments were carried out with *sterilised* soils.

The influence of the *saprophytic bacteria in the soil* on the life of pathogenic organisms is now being investigated in Professor Macfadyen's laboratory, and the results will form a sequel to those which I have detailed.

The filtering experiments showed that when the soils were used in a *pure* state moist yellow sand proved the most efficient filter for the micro-organisms, whilst peat not only retained but killed the bacilli.

(For report of the discussion on this paper, see 'Proceedings of the Royal Medical and Chirurgical Society,' Third Series, vol. vi, p. 141.)