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EXPERIMENTS MADE TO DETERMINE THE CONDITIONS UNDER WHICH "SPECIFIC" BACTERIA DERIVED FROM SEWAGE MAY BE PRESENT IN THE AIR OF VENTILATING PIPES, DRAINS, INSPECTION CHAMBERS AND SEWERS.[1]

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MOST sanitarians at the present time believe that when sewage is in a putrefactive condition and gas bubbles rising through it are bursting at the surface, bacteria may be carried into the air of drains and sewers. It is also considered possible that when sewage has dried on the surfaces of pipes, bacteria may be separated as dried particles and carried some distance by currents of air passing through the pipes.

The following experiments were designed to ascertain whether there are any scientific facts on which to base these beliefs, and may be arranged in three groups.

GROUP 1.—*Experiments to determine whether Specific Bacteria are ejected into the Air by the bursting of Bubbles at the surface of Sewage.*

In the first series of experiments, sewage obtained from a main sewer in Gibraltar was inoculated with a rich emulsion of *Bacillus*

[1] Reprinted from the *Proceedings of the Royal Society* of May 9th, 1907.

prodigiosus and then poured into a deep glass jar so as to form a layer at the bottom about 2 inches deep. At a height of 4 inches from the surface of the sewage two Petri dishes containing nutrose-agar were fastened, the medium facing upwards, to wire tripods, which were then firmly wedged in the bottom of the dish. A glass cover was then put on the dish and the fluid gently shaken by a horizontal movement, until a layer of bubbles formed on the surface of the sewage. This procedure was followed at intervals for three days, the plates were then taken out and incubated at 22° C. After seven days' incubation no signs of the *B. prodigiosus* appeared. Only one colony composed of cocci derived from the air was seen. This

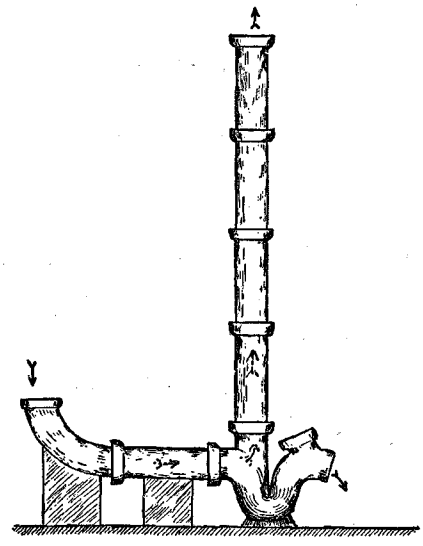


FIG. 1.

experiment was repeated again and again, but invariably with negative results.

The sewage was next inoculated with a rich emulsion of *B. typhosus*, and litmus-lactose-nutrose-agar plates were fastened to the tripods. The dish was shaken as before, the plates were then removed and incubated at 37° C. No signs of *B. typhosus* or *B. coli* were observed after incubation for one week. Old sewage, smelling strongly of sulphuretted hydrogen gas, was then placed in the dish and the experiments were repeated as before. It was thought that gas bubbles would form more readily in old than in fresh sewage; this proved to be the case, but the special organisms added to the sewage never appeared in the plates.

Soapy water from a lavatory basin was then inoculated with *B. prodigiosus* and freely shaken in a glass bottle until it was permeated with bubbles; the fluid was then transferred to the glass dish, the plates fastened to tripods were rapidly placed *in situ*, and the cover put on. Twenty-four hours elapsed before all the bubbles dispersed; the plates were then taken out and incubated as before, but no signs of the *B. prodigiosus* appeared.

In the above experiments there were no air currents circulating above the sewage, and the bacteria could only be ejected by the bursting of bubbles. The results appear to show that independently of air currents, bacteria will not be ejected to a height of 4 inches by the bursting of infected bubbles.

The next series of experiments were made with the apparatus

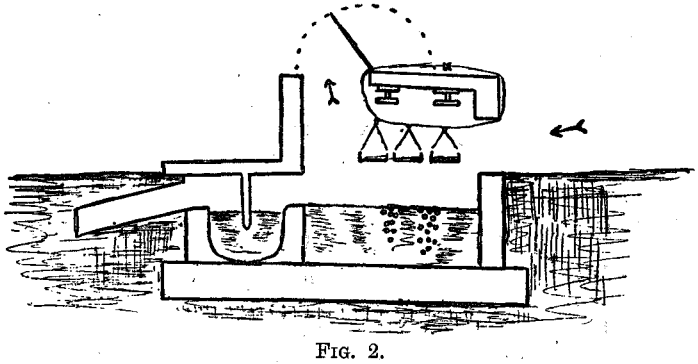


FIG. 2.

shown in fig. 1. Plates of nutrose-agar were suspended by means of wire cages in the vertical pipe, the uppermost plate being 9 feet above the water in the trap. Soapy water, inoculated with an emulsion of *B. prodigiosus*, was shaken up in a glass stoppered bottle until the whole fluid was permeated with bubbles; the contents of the bottle were then poured into the trap until it was filled. Under these conditions currents of air passing up the vertical pipe were able to carry bacteria separated from the fluid by the bursting of bubbles. The plates were removed at the end of two hours and incubated at 22° C. The result was that colonies of *B. prodigiosus* appeared in every plate at the end of seventy-two hours.

The last experiment of this series was made with a catch-pit on the storm-water system of the town. Complaints having been made of foul odours arising from the pit, the hinged cover was thrown back, and it was then seen that bubbles were rising through

the water retained in the pit as a result of fermentation processes going on in the mud at the bottom. The pit contained 180 gallons of surface water at the time. An emulsion of *B. prodigiosus* was poured into the water and plates of nutrose-agar were then suspended as shown in fig. 2. Twenty-four hours later the plates were removed and incubated at 22° C. for two days, when numerous colonies of *B. prodigiosus* were found in all the plates. Control plates of nutrose-agar, exposed to the air outside the pit, did not show any signs of the special organism used in the experiment.

These results show that bubbles rising through stagnant water may eject bacteria, which will be carried away by currents of air passing over the surface of the fluid.

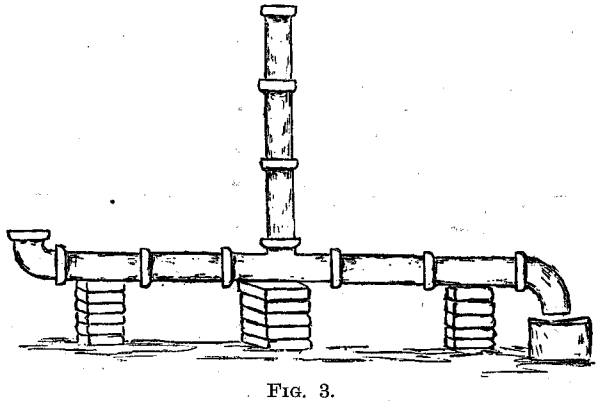


FIG. 3.

GROUP 2.—*Experiments to determine whether Bacteria dried on the surfaces of Pipes are likely to be separated and carried by Currents of Air passing through the System.*

The apparatus shown in fig. 3 was employed. Three 2-ft. lengths of 6-inch piping were laid on the ground and a rich emulsion of *B. prodigiosus* was poured inside each of them. The pipes were then rolled backwards and forwards until the fluid was uniformly diffused over the inner surface of each pipe. Twenty-four hours later the pipes being perfectly dry were fitted together with clay joints so as to form the vertical pipe of fig. 3. Nutrose-agar plates were then suspended in the pipe and sewage was allowed to flow through the horizontal piping at a rate not exceeding 3 feet per second for twenty minutes. The plates were then removed and incubated at 22° C.

At the end of seventy-two hours, all the plates were found studded with colonies of *B. prodigiosus*. The experiment was repeated several times, and on each occasion the same result was obtained. As the sewage passing through the horizontal pipes did not contain *B. prodigiosus*, and control plates exposed to the air were also free from this organism, it is fair to assume that the currents of air produced by the passage of sewage through the horizontal pipes carried up dried particles of *B. prodigiosus* detached from the walls of the vertical pipe.

GROUP 3.—*Experiments to determine whether Specific Bacteria are ejected into the Air of Drains, Sewers, &c., from Sewage flowing under Normal Conditions.*

In the first series of experiments, the trap of a 6-inch disconnecting trap was filled with sewage, and two lengths of 6-inch drain

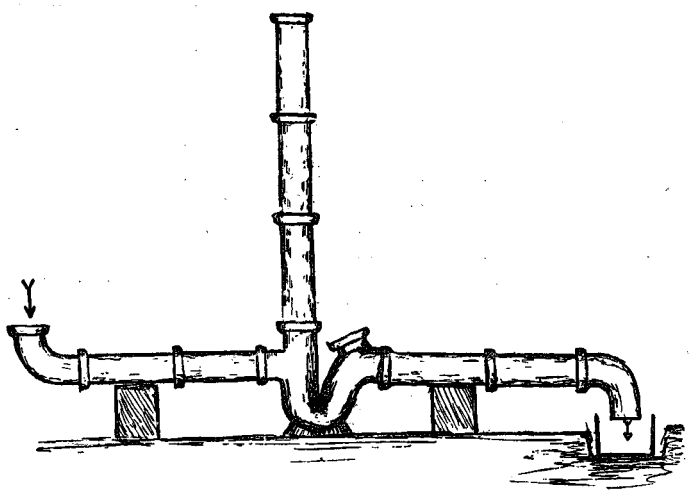


FIG. 4.

piping, having a junction bend turned upwards, fixed at one end, were fitted horizontally into the house side of the trap. Similar lengths of drain piping, but with the junction bend turned downwards, were also fitted to the sewer side of the trap. The vertical portion above the trap was lengthened by the addition of three 2-ft. lengths of 6-inch drain piping. The apparatus is shown in fig. 4.

Three litmus-lactose-nutrose-agar plates were next suspended

with the media facing upwards in the vertical pipe by means of wire cages, the uppermost plate being about 6 feet 9 inches above the sewage in the trap. Three gallons of sewage, taken from a main sewer in Gibraltar, were then inoculated with a rich emulsion of the *B. typhosus*, and poured down the bend on the house side of the trap. The sewage passed through the apparatus at a rate not exceeding 3 feet per second, and was received in a bucket placed under the bend on the sewer side of the trap. The inoculated sewage was passed through the trap in the same manner on two successive days for about half an hour. The plates were then removed and inoculated at 37° C. for twenty-four hours, when numerous transparent blue colonies, resembling those of the *B. typhosus*, were seen in each plate. Several of the colonies were tested with anti-typhoid serum (horse) diluted 1—100, and, agglutination occurring at once, the colonies were plated out on agar slopes. The growths thus obtained were examined as to morphology and Gram staining, and then plated out in the usual media. The following results were obtained :—

Medium.	Result of incubation at 37° C.
Glucose-peptone	Acid, no gas.
Lactose-peptone	Unchanged.
Maltose-peptone	Acid, no gas.
Cane-sugar peptone	Unchanged.
Starch-peptone	Unchanged.
Neutral-red	Unchanged.
Litmus-milk	Very faint acidity, no clotting.
Peptone-water	No nitroso-indol reaction.
Potato	Colourless growth.
Proskauer and Capaldi, No 1	No growth.
Gelatine	Thin transparent growth, medium not liquefied.
Nitrate broth	Reduced to NO_2.
Morphology, &c.	Small motile bacillus.
Gram staining	Decolorised.

The bacilli were agglutinated by an anti-typhoid horse serum diluted 1—500.

A portion of the agar growth derived from one colony was then emulsified in water and injected subcutaneously into a guinea-pig. As a control, a similar emulsion of the *B. typhosus* used to inoculate sewage was injected into a second guinea-pig of approximately the same weight. At the end of three weeks the sera of both guinea-pigs agglutinated, in a dilution of 1—100, the stock culture of *B. typhosus*.

It is plain that in this experiment the *B. typhosus* was ejected from the sewage to a height of 6 feet 9 inches.

Further experiments on the same lines were then carried out with the *B. prodigiosus*, an agar growth emulsified in water being added to the sewage. The same apparatus was used as in the previous experiments, the vertical pipe being gradually lengthened by the addition of 2-ft. lengths of 6-inch piping. The *B. prodigiosus* was recovered from the plates suspended 8 feet 9 inches and 11 feet 9 inches above the sewage in the trap.

It was thought that possibly the resistance produced by the passage of the sewage through the trap might have caused the projection of the special bacteria employed into the air contained in the vertical pipe.

Accordingly, in the next series of experiments the trap was removed and the apparatus fitted up as shown in fig. 3. Plates of nutrose-agar were suspended in the vertical pipe, and the sewage mixed with *B. prodigiosus* was made to flow at a rate not exceeding 3 feet per second through the horizontal pipes, which were never more than half filled with the sewage. The special organism was again recovered from plates suspended 11 feet 9 inches above the sewage in the trap.

As in all the above experiments a very rich emulsion of the special organism, such as would never be found under natural conditions, was added to the sewage, it was determined to repeat the experiments, employing only 1 cc. of the emulsion, representing one-ninth of the growth on an agar slope after forty-eight hours' incubation at 22° C., to inoculate the sewage. The same results were obtained as when the rich emulsion was used.

It is evident from these experiments that special bacteria can be ejected from flowing sewage independently of the resistance offered to the flow by the disconnecting trap. But as the plates were left in the vertical pipe for more than twenty-four hours, it is possible that the colonies in the plates might have been derived from particles dried on the surfaces of the pipes. In order to exclude this source of the bacteria, the experiments were repeated again, but the plates were withdrawn immediately the sewage had ceased to flow, each experiment only occupying twenty minutes. As before, colonies of *B. prodigiosus* were found in all the plates, showing that they must have been produced independently of dried particles carried by currents of air. As there were very few bubbles visible to the naked eye in the flowing sewage, it is not very probable that all the microbes found in the plates were ejected into the air by the bursting of bubbles. I think it is possible that many of the colonies were caused by the ejection of minute infected droplets

from the flowing sewage. Gross splashing is out of the question, as the sewage was flowing at a comparatively slow rate, and plates were found infected at a height of 11 feet 9 inches above the surface of the fluid.

The next series of experiments were undertaken to test the value of the disconnecting trap as a means of protecting a house drainage system from specific bacteria present in the air of the sewer into which the house drain discharges.

The apparatus employed is shown in fig. 5. It was a combination of the arrangements used in the experiments already described. The straight run of piping, with the vertical pipe attached to it, represents the sewer and an attached ventilating pipe; joining the sewer is the house drain, the air in which is separated from that in the sewer by the usual disconnecting trap. The vertical pipe

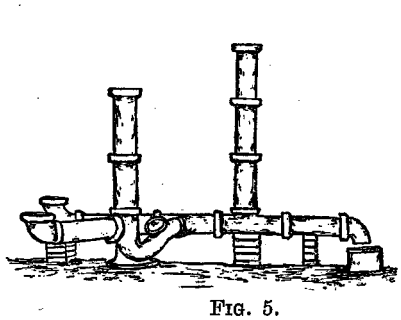


FIG. 5.

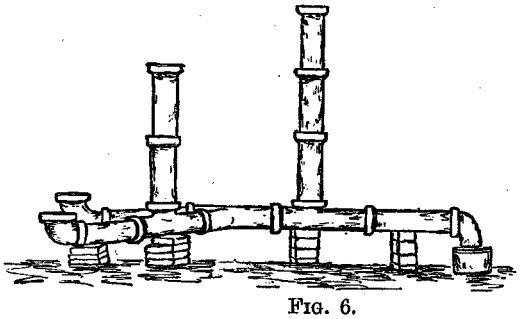


FIG. 6.

above the trap, represents the air inlet or outlet, as the case may be, of the house drainage system. Plates of nutrose-agar were suspended in both vertical pipes. Sewage inoculated with *B. prodigiosus* was then allowed to flow at a rate not exceeding 3 feet per second through the pipes representing the sewer. The flow of sewage was continued for half an hour on two successive days; the plates were then withdrawn and incubated at 22° C. Colonies of *B. prodigiosus* were found in the plates placed in the ventilating pipe of the sewer, but none were present in the plates placed in the ventilating pipe above the disconnecting trap.

The experiment was repeated again, but during the flow of the infected sewage through the sewer the disconnecting trap of the house system was repeatedly flushed with three gallons of sewage. It was thought that during the flushing of the trap, microbes in the sewer air might possibly enter the house drainage system. This never occurred, the plates on the house side of the disconnecting trap never showed any colonies of *B. prodigiosus*.

The vertical trap was then removed and the apparatus arranged as shown in fig. 6.

Inoculated sewage was made to flow through the sewer as before, and every five minutes a three-gallon flush was passed through the house drain. Under these conditions colonies of *B. prodigiosus* appeared in the ventilating pipe of the house system as well as in that of the sewer. These results show that a disconnecting trap prevents microbes present in the sewer air from passing into the house drainage system.

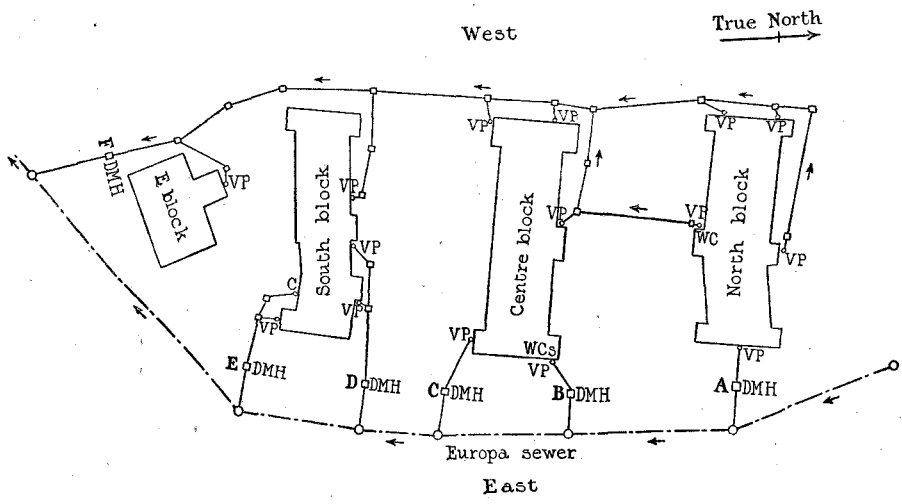
The next series of experiments were made with actual drainage systems.

In the first experiment a newly laid system of a house in the town was tested by suspending plates in the junction and disconnecting chambers. A plate was also fastened in front of the air inlet of the system, the air inlet being provided with a mica valve. An emulsion of *B. prodigiosus* was placed in the pan of the W.C. of the house, which was then flushed out. The plates were removed twenty-four hours later and incubated, with the result that colonies of *B. prodigiosus* were found in every plate. "Air" plates exposed showed no signs of the special organism used in the experiment.

The second experiment was made at the military hospital, plates being suspended in the top of a 6-inch ventilating pipe connected with a W.C. in the basement. The plates were 50 feet above the ground level. A rich emulsion of *B. prodigiosus* was placed in the pan of the W.C. and the contents were then flushed in the usual manner. At the end of twenty-four hours the plates were removed and incubated for forty-eight hours, when all the plates were found studded with colonies of the special organism.

The third experiment was made in connection with the drainage at the east end of the Centre Block of the military hospital. At the north-east corner of the block there is a ventilating soil pipe, 6 inches in diameter, receiving the contents of a row of W.C.'s placed in an annexe on the first floor of the hospital. The soil pipe is connected by a 6-inch drain to a disconnecting chamber (B), which is 10 feet deep, and discharges its contents by a short drain into a 9-inch sewer running along the Europa Road. At the south-east corner of the block there is a similar disconnecting chamber (C), which receives the drainage from a W.C. in the basement and is also connected to the Europa sewer. There are six inspection chambers, with ventilating covers at the road level, and a 6-inch ventilating pipe on the Europa sewer, between the Centre Block and the Europa Pass Barracks, a distance of some 200 yards (see

Plan of hospital drainage). Plates were suspended in the top of the hospital soil pipe, at the north-east corner, some 30 feet above the junctions of the W.C.'s, in the disconnecting chambers (A), (B), and (C), in all the inspection chambers connected with the Europa sewer and in the top of the ventilating pipe of the sewer. A rich emulsion of *B. prodigiosus* was then placed in each of the pans of the W.C.'s, which were then flushed. Twenty-four hours later the plates were removed and incubated at 22° C. After forty-eight hours all the plates, except those in disconnecting chamber (A), were found studded with colonies of the special organism employed. Plates exposed on the ground close to the Centre Block for twenty-



four hours were also incubated, but no signs of the *B. prodigiosus* appeared in them.

Judging by the results obtained with the experimental installations, it was expected that the special organism would be found in the hospital soil pipe, disconnecting chamber (B), and in the chambers and ventilating pipe of the sewer. But as the disconnecting chamber (C) at the south-east corner of the Centre Block is not in any way connected with the W.C.'s receiving the emulsion of the *B. prodigiosus*, and is separated from the Europa sewer by a modern disconnecting trap, the special organism should not have appeared in this chamber. The experiment was repeated again three times, and, in addition to the places already mentioned, plates were also suspended in the disconnecting chambers (D) and (E)

connected to the east side of the South Block. The plates were left *in situ* for twenty-four hours, and then incubated as before. Colonies of *B. prodigiosus* appeared in the same places as in the previous experiment, but none were found in the chambers (D) and (E).

It was then evident that there must be some means by which the disconnecting chamber (C) was placed in communication with the Europa sewer. A careful examination of the bottom of the chamber was made, and it was found that there was no plug in the cleaning arm of the disconnecting trap; consequently, the chamber was in direct communication with the air in the sewer. Plugs were found firmly fixed in the cleaning arms of the disconnecting traps in the chambers (D) and (E), and prevented micro-organisms in the sewer air from entering the chambers.

The cleaning arm in the chamber (C) was then firmly plugged and the experiment repeated. The result was that colonies of *B. prodigiosus* appeared in the plates placed in the hospital soil pipe and in the chamber (B), but none appeared in the plates suspended in chamber (C), proving conclusively that the patent cleaning arm had been the channel through which the *B. prodigiosus* entered this chamber in the previous experiments.

As a control of the above experiments, plates were next placed in the inspection chambers connected with the west end of Centre and South Blocks. The contents of these chambers are removed by a separate drain, which passes along the west of the hospital into the disconnecting chamber (F), close to the south gate of the hospital. No colonies of *B. prodigiosus* were found in any of the chambers, although the plates were treated precisely as in the previous experiments. A fortnight later the experiment was repeated, but on this occasion the pan of a w.c. connected with the drain on the west side of the hospital was inoculated with *B. prodigiosus*, and the w.c.'s on the east side were left in their natural condition. The result was that colonies of the special organism appeared in the plates placed on the west of the hospital, but none were found on the east side.

In all the above experiments the plates were left in the chambers and soil pipes for twenty-four hours, consequently the *B. prodigiosus* might have resulted from dried particles of the growth separated from the walls of the pipes and chambers and carried upwards by air currents, as well as from the bursting of bubbles and ejection of droplets from the flowing sewage. In order to determine whether the separation of dried particles was the source from which

the bacteria were obtained, the experiments were repeated, but on these occasions the plates were withdrawn as soon as the W.C.'s had been flushed, each experiment only taking twenty minutes. When incubated the plates showed practically as many colonies of the *B. prodigiosus* as in the previous experiments, showing that special bacteria may appear in the air of the chambers and pipes independently of the separation of dried particles.

It might be objected that in the experiments just related the bacteria were suspended in water and had no organic matter adhering to them such as would be the case under natural conditions. When fæcal material is flushed down W.C.'s and carried through a drainage system, it is probable that the *B. coli* and the *B. typhosus* in the case of typhoid stools, will have an organic envelope which may materially affect the results.

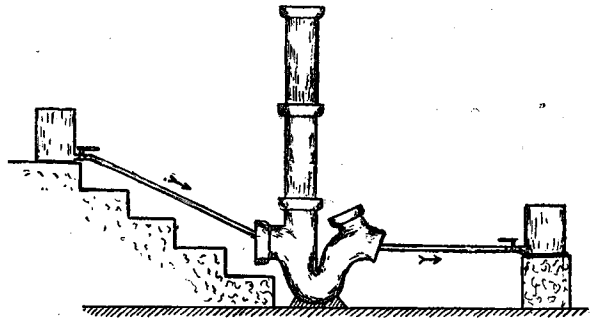


FIG. 7.

In order to ascertain whether the objection raised had any force, plates of litmus-lactose-nutrose-agar were suspended in the ventilating pipe of the Centre Block for twenty-four hours and then incubated. All colonies having a reddish tint were fished and carefully studied, the result being that typical members of the coli group were readily isolated. Plates were also suspended in large inspection chambers connected with one of the main sewers of the town, about ten feet above the flowing sewage. The plates were removed at the end of four hours and incubated, when typical members of the coli group were again readily isolated. As a final test, the possibility of the *B. typhosus* being ejected from typhoid stools was ascertained by using the apparatus shown in fig. 7. The trap was filled with sewage, and litmus-lactose-nutrose-agar plates, the media facing upwards, were suspended in the vertical pipe, which was afterwards covered with a glass plate. A typical stool, obtained from a case of enteric fever under treatment in the military hospital, was then

mixed with two gallons of water placed in the can connected by indiarubber piping, 1·5 inches in diameter, with the house side of the trap. The taps were next opened, and the contents of the can having passed slowly through the trap, were collected in the can connected to the P outgo of the trap. The taps were then turned off and, the cans having been changed, the infected sewage was again passed through the trap. This procedure was followed until the sewage had passed twelve times through the trap. The plates were then removed and incubated. Next day several transparent blue colonies were observed, these were fished and planted on agar slopes. The growths resulting were tested with an anti-typhoid horse serum, and one was found to be completely agglutinated by the serum diluted 1—500. The growth was then submitted to the usual tests, which showed that under the conditions mentioned a true *B. typhosus* had been carried up the vertical pipe.

Two days later the experiment was repeated with another stool from the same patient, with the result that three colonies of *B. typhosus* were isolated, one being in a plate 2 feet above the trap and the other two in a plate 3 feet 6 inches above the trap.

The experiments were so conducted that no splashing could possibly occur, and on looking through the glass plate on the top of the vertical pipe, when sewage was flowing through the trap, a few bubbles were seen bursting at the surface of the fluid. The pipes and trap employed were quite new, and had not been used in any of the previous experiments.

These results show that bacteria existing in sewage under natural conditions can be ejected into the air in the same manner as the naked bacteria used in the experiments already recorded:

Conclusions.

The experiments show that :—

(1) Specific bacteria present in sewage may be ejected into the air of ventilation pipes, inspection chambers, drains, and sewers by (*a*) the bursting of bubbles at the surface of the sewage; (*b*) the separation of dried particles from the walls of pipes, chambers, and sewers, and probably by (*c*) the ejection of minute droplets from flowing sewage.

(2) A disconnecting trap undoubtedly prevents the passage of bacteria, present in the air of a sewer, into the house drainage system.

(3) An air inlet, even when provided with a mica valve, may be a source of danger when it is placed at or about the ground level.