ON THE PROTECTION FROM WATERBORNE DISEASE AFFORDED BY THE PASTEUR- CHAMBERLAND AND BERKEFELD FILTERS.

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MANY investigations have been made with the object of ascertaining the extent and duration of the protection from waterborne disease afforded by the Pasteur-Chamberland and Berkefeld filters. Experiments soon showed that the *direct* passage of micro-organisms through the candles of these filters, consequent on some flaw in the material or imperfection in the fittings, must be distinguished from the *indirect* passage of bacteria, consequent on the gradual growth of the organisms through the lacunar spaces of the filter material. The direct passage is easily tested by means of some colour-producing organism, such as the *B. prodigiosus*, and all the experiments made with Pasteur-Chamberland and Berkefeld filters show that the direct passage of bacteria is prevented, provided the flow through the candles is steady and continuous. If the flow be intermittent, there appear to be strong reasons for believing that with each action of an attached pump the direct passage of micro-organisms will take place. This intermittent action is easily obviated by introducing an air chamber between the pump and the filtering candle.

The indirect passage of pathogenic and non-pathogenic micro-organisms through the Pasteur-Chamberland and Berkefeld candles has been the subject of much experiment. Most observers now admit that water bacteria eventually grow through these candles; but with regard to the typhoid bacillus and the spirillum of cholera there is not quite the same unanimity of opinion. Freudenreich's experiments appeared to show that the typhoid bacillus, even when present in a broth culture, did not grow through the walls of a Pasteur-Chamberland candle. Kirchner demonstrated that when broth cultures of cholera vibrios and typhoid bacilli were filtered through Berkefeld candles, the vibrios appeared in the filtrate after twenty-four hours, and the typhoid bacilli after seventy-two hours. Gruber criticised Kirchner's work, and pointed out that in the filtration of water only those bacteria which were able to multiply in the pores of the filtering candle could appear in the filtrate. He considered that ordinary drinking water would not supply sufficient food material to enable pathogenic organisms to multiply in this manner. Schöfer's experiments seemed to prove conclusively that typhoid bacilli, inoculated into pure and impure waters, would not grow through the walls of a Berkefeld candle. He found, however, that the addition of a little broth to natural waters caused the typhoid bacilli to appear in the filtrate after the lapse of forty-eight hours. Sims Woodhead and Cartwright Wood repeated Schöfer's experiments. They arrived at the conclusion that waters naturally highly contaminated, and even sewage itself, did not appear to supply pathogenic organisms with a sufficiently favourable pabulum to enable them to traverse the walls of Pasteur-Chamberland and Berkefeld candles. They also found that the addition of a little broth to infected waters caused the appearance of pathogenic organisms in the filtrate.

The results of the various experiments hitherto made being somewhat contradictory, it seemed desirable, in view of the importance of the subject, to reinvestigate the indirect passage of pathogenic organisms through Pasteur-Chamberland and Berkefeld candles. The following experiments were devised in order to give the typhoid bacillus the maximum chance of growing through the candles, and to supply a medium in which it would readily manifest its presence, should it succeed in traversing the filtering materials.

Experiment I.—A Berkefeld candle, No. 12 in the catalogue, fitted with a glass mantle, was firmly fixed in position by means of a binding screw. The metal delivery pipe was then connected by means of a short piece of stout indiarubber tubing, previously fitted with a pinchcock, to a short glass tube which just passed through an indiarubber cork, fitted tightly into a Kitasato flask. The arm of the flask and the mouth of the glass mantle were plugged with cotton wool. The whole apparatus was then

placed in the steriliser and steamed for an hour. The apparatus having been allowed to cool, the filtering candle and mantle were connected in the upright position to an ordinary burette stand, and 50 c.cm. of sterile broth were then introduced into the flask, with every precaution. The pinchcock being placed in position on the indiarubber tube so as to separate the contents of the candle and mantle from the flask, all the joints of the apparatus were made watertight with melted paraffin wax. The mantle was then filled with broth so as to completely cover the filtering candle without touching the cotton wool. By opening the pinchcock 10 c.cm. of the broth from the mantle were now allowed to filter through the bougie into the flask. The filtration occurred simply as the result of atmospheric pressure, and the bougie worked under conditions which would allow its molecular influence full play. The apparatus was then placed in a warm cupboard, the temperature of which averaged 25° C. After forty-eight hours the broth in the mantle and flask was found perfectly clear, showing that the manipulations had been carried out without introducing any contamination. The broth in the mantle was then inoculated with a loopful of a twenty-four hours' agar culture of *B. typhosus*. Next day the broth in the mantle was found uniformly turbid, but the broth in the flask was perfectly clear; the pinchcock was then opened, and 10 c.cm. of the contents of the mantle were allowed to filter through into the flask. On the following day the broth in the flask being still perfectly sterile, 10 c.cm. were again filtered through from the mantle. The same filtration process was carried out day by day, and the amount of broth filtered each day from the mantle was replaced by sterile broth, the manipulation being carried out with every precaution. The broth in the Kitasato flask remained perfectly clear for four days; on the fifth day of filtration the broth in the flask was found slightly turbid, a loopful was then withdrawn by means of a platinum wire passed through the arm of the flask, and planted out on an agar slope. The thin greyish growth which resulted was then subcultured and tested for agglutination. The culture of *B. typhosus* used for the inoculation of the mantle was also tested at the same time. The results were as follows:

	B. Typhosus in the Mantle.	Micro-organism in the Filtrate.
Agglutination with Berne antityphoid serum	Complete 1 in 1,000 Marked 1 in 10,000	Complete 1 in 1,000 Marked 1 in 10,000
Potato	Thin colourless growth	Thin colourless growth
Milk (three weeks at 37° C.)...	Unchanged	Unchanged
Proskauer and Capaldi No. 1 medium	No growth	No growth
Proskauer and Capaldi No. 2 medium	Growth, medium acid	Growth, medium acid
Peptone and salt solution ...	No indol reaction	No indol reaction
Litmus whey (seven days at 37° C.)	Acidity equal to 5.4 per cent. N alkali 10	Acidity equal to 5.3 per cent. N alkali 10
Glucose gelatine, lactose gelatine	No gas formation	No gas formation
Gelatine stab and plates ...	Very slow growth	Very slow growth
Motility	Very motile	Very motile
Stained by Gram	No	No.

The organism which appeared in the filtrate was evidently the *B. typhosus* which in five days had grown through the walls of the No. 12 filtering candle. This result is in harmony with the experiments of Schöfer and Sims Woodhead.

Experiment II.—The same form of apparatus as in Experiment I was employed, a new Berkefeld bougie (No. 12) being fixed in the mantle. Fifty c.cm. of sterile broth having been placed in the Kitasato flask, and all the joints made tight with melted paraffin, the mantle was filled with a sample of sterile barrack sewage. The specimen of sewage on chemical analysis gave the following results expressed as parts per 100,000: Total solids, 610; chlorine, 52; free ammonia, 1.44; albuminoid ammonia, 1.846; oxygen absorbed (Tidy, four hours), 8. By releasing the pinchcock 10 c.cm. of the sterile sewage were allowed to filter through into the broth; the apparatus was then incubated at 25° C. Next day the broth in the flask being perfectly clear, the sewage in the mantle was inoculated with the same agar culture of *B. typhosus* as was employed for the first experiment. Ten c.cm. of the infected sewage were then filtered into the broth every twenty-four hours, the amount filtered from the mantle each day being replaced by sterile sewage. The broth in the flask remained perfectly clear until the fifth day, when a distinct turbidity was noticed; a loopful of the turbid broth was withdrawn through the arm and planted out on agar. The growth of bacilli which resulted was tested, as in Experiment I, and gave the following results: *Agglutination*: The bacillus was completely agglutinated by Berne serum diluted 1 in 1,000, and partially agglutinated by the serum diluted 1 in 10,000. *Potato*: A moist transparent growth. *Peptone solution*: No indol produced. *Milk*: Unchanged. *Glucose-gelatine* (shake): No gas formation. *Litmus-whey*: Acidity equalled 4 per cent. N alkali. *Proskauer and Capaldi's media*: No. 1, no growth; No. 2, rendered acid. *Gelatine Plates*: Growth slow, colonies small and resembling those of *B. typhosus*. *Microscopical appearance*: A small, highly-motile bacillus, decolorised by Gram.

The tests showed that the bacillus which appeared in the filtrate was identical with the *B. typhosus* planted out in the sewage in the mantle. As the fittings were absolutely watertight and no leakage could possibly occur from the mantle into the Kitasato flask, and as the bacillus did not appear in the filtrate until the fifth day, this experiment appeared to show that the sewage, just like the broth, had supplied suffi-

cient nutriment to enable the typhoid bacillus to grow through the walls of the filtering candle.

Experiment III.—The same form of apparatus was employed as before, and the mantle was filled with sterile barrack sewage largely diluted with distilled water. The dilute sewage was found to have the following composition, expressed as parts per 100,000: Total solids, 7; chlorine, 0.3; oxygen absorbed (Tidy, 4 hours), 1.1; free ammonia, 0.1248; albuminoid ammonia, 0.2016; nitrites, absent; nitrates (NO_3), 0.926. The dilute sewage was inoculated with a subculture of the *B. typhosus* employed in the former experiments; filtration was then carried on daily, the amount filtered being replaced by sterile sewage so that the filtering candle was never allowed to become dry or exposed to the air. The broth in the flask remained perfectly clear for nine days, but on the morning of the tenth day of filtration a slight turbidity was noticed. A loopful of the turbid broth was removed and planted out on agar.

The growth which resulted was found to be a pure culture of *B. typhosus*. The results of the agglutination and cultural tests exactly corresponded with those of the *B. typhosus* introduced into the mantle. In this experiment the typhoid bacillus was not supplied with as much nutriment as in the two former experiments, and it appeared to require nine days to grow through the walls of the filtering candle.

Experiment IV.—In this experiment sterilised surface water from a ditch was placed in the mantle. The water on analysis gave the following results, expressed as parts per 100,000: Total solids, 44; volatile solids, 16; chlorine, 6.2; free ammonia, 0.526; albuminoid ammonia, 0.60; oxygen absorbed (Tidy, 4 hours), 2.2; nitrites, absent; nitrates (NO_3), 1.005. The water in the mantle was inoculated with a loopful of an agar culture of *B. typhosus*, and filtration was then carried on daily as in the previous experiments. The broth in the flask remained perfectly clear for eight days, but on the ninth day distinct turbidity was noticed.

A loopful of the turbid broth, when examined as before, was found to contain a pure culture of *B. typhosus*.

Experiment V.—This was simply a repetition of Experiment II, a new No. 12 bougie and a fresh sample of sewage being employed. The results of the chemical analysis of the barrack sewage, after sterilisation, were as follows: Total solids, 55; chlorine, 0.5; free ammonia, 8.28; albuminoid ammonia, 5.07; oxygen absorbed (Tidy, 4 hours), 6.4; nitrites and nitrates, absent. The mantle was inoculated with a loopful of an agar culture of *B. typhosus*, and filtration carried on as in the previous experiments.

The typhoid bacillus was found in the filtrate on the fifth day. The available organic matter was much greater in this experiment than in III and IV, and the bacillus only required four days to grow through the walls of the candle.

Experiment VI.—The same form of apparatus was employed, a new No. 2 candle being fitted into the mantle. A specimen of water collected from a reservoir in Netley was sterilised, and then analysed. The results obtained were as follows, expressed in parts per 100,000: Total solids, 18; chlorine, 3.3; free ammonia, 0.00816; albuminoid ammonia, 0.0158; oxygen absorbed (Tidy, four hours), 0.5; nitrites, nil; nitrates (NO_3), 0.8. This water was placed in the mantle, and inoculated with an agar culture of *B. typhosus*. Filtration was carried on as in the previous experiments, and the broth in the Kitasato flask remained perfectly sterile until the ninth day, when slight turbidity was noticed. A loopful of the turbid broth was planted out on agar, and tested in the usual manner.

A pure culture of *B. typhosus* was obtained.

Experiment VII.—The same water was used for this experiment, which was conducted in exactly the same manner as Experiment VI, a new, sterile No. 12 candle being employed.

The typhoid bacillus appeared in the filtrate on the ninth day.

Experiment VIII.—Candle, No. 10, fixed in a large glass mantle, was attached to a large Kitasato flask with the usual precautions. Sterile reservoir water was placed in the mantle, inoculated with *B. typhosus*, and then filtration carried on as in the previous experiments.

The broth in the flask remained perfectly sterile for ten days; on the eleventh day a growth appeared, which was found to be caused by *B. typhosus*.

Experiment IX.—A perfectly new No. 10 candle fixed in a mantle was employed. The mantle was filled with sterile barrack sewage, having the same chemical composition as that used in Experiment V.

On the fifth day of filtration the typhoid bacillus was found in the filtrate.

Experiment X.—The candle used in Experiment VI was removed from the mantle and thoroughly cleaned with a stiff brush; the metal delivery pipe was also thoroughly washed and flamed. The candle was then replaced in a sterile mantle, and the apparatus fitted up in the usual manner. Sterile reservoir water was then filtered through the candle; next day the broth in the Kitasato flask was found quite turbid.

The typhoid bacillus was easily isolated from the turbid broth. This result appeared to show that carefully cleaning a candle would not remove the *B. typhosus*, which had evidently penetrated into the interior of the filtering material, and then been washed through into the filtrate by the passage of the sterile water.

Experiment XI.—The candle used in Experiment VII was sterilised with boiling water, and then fitted up in a sterile apparatus. Sterile reservoir water was then filtered through the candle for eleven days.

The broth in the flask remained perfectly clear, and loopfuls withdrawn and planted out on agar produced no growth.

Experiments on the same lines were then made with Pasteur-Chamberland candles, fitted into glass mantles by

means of indiarubber bungs. The delivery pipe of the candle in each apparatus was connected by a short piece of india-rubber tubing, fitted with a pinchcock, to a Kitasato flask containing sterile broth. All the joints were then made watertight with melted paraffin wax. In the first experiment the mantle was filled with sterile broth, which was inoculated with a twenty-four hours' agar culture of *B. typhosus*. Next day the broth in the mantle was extremely turbid. Filtration was then commenced, 10 c.cm. of broth being filtered into the flask every day, and the amount filtered replaced by sterile broth in the mantle. After filtration for three weeks the broth in the flask was found quite sterile. One loopful of the broth from the mantle, however, contained a very large number of typhoid bacilli.

Trials were then made with other candles, fitted up in the same manner. Sterile barrack sewage, sterile sewage effluents, polluted ditch water, and reservoir water, repeatedly inoculated with large doses of *B. typhosus*, were filtered for three weeks, but no traces of the typhoid bacillus could be detected in the filtrates. The failure to pass through the filtering candle was not due to the death of the typhoid bacilli in the fluid contained in the mantle, as in each experiment one loopful of the fluid from the mantle, taken at the end of the trial, was found to contain a very large number of living typhoid bacilli.

The following conclusions appear justified:

1. Typhoid bacilli are not able to grow through the walls of a Pasteur-Chamberland candle, and if proper care be taken to prevent the direct passage of the bacilli through flaws in the material or imperfections in the fittings, the Pasteur-Chamberland filter ought to give complete protection from waterborne enteric fever.

2. Typhoid bacilli can grow through the walls of Berkefeld candles, probably owing to the larger size of the lacunar spaces and the consequently diminished immobilising and devitalising influences. The time required for the typhoid bacilli to traverse a candle varies between four and eleven days, and appears to be largely dependent on the nutriment supplied to the organisms by the medium in which they exist. In order to obtain complete protection from waterborne enteric fever, when employing Berkefeld filters, it is necessary to sterilise the candles in boiling water every third day.

REFERENCES

- ¹ Freudenreich, *Centralb. f. Bakt.*, vol. xii, No. 7-8, p. 240. ² Kirchner, *Zeit. f. Hyg.*, vol. xiv, p. 299. ³ Gruber, *Centralb. f. Bakt.*, vol. xiv, p. 488. ⁴ Schöfer, *Ibid.*, vol. xiv. ⁵ Woodhead and Wood, *BRITISH MEDICAL JOURNAL*, January, 1898.

HÆMATURIA FOLLOWING THE ADMINISTRATION OF UROTROPIN.

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THE use of urotropin in enteric fever has been advocated of late by several authorities. Horton-Smith¹ urges the administration of 30 grs. daily in all cases throughout the whole course of the fever and during the first three weeks of convalescence, as first suggested by Richardson. In this way he considers it may be possible to prevent urinary complications in the patient, and also the spread of infection to others through the urine. He adds that, taken in such doses and during such a prolonged period, the drug produces no ill-effect beyond some urethral pain occasionally if the urine be allowed to become concentrated. Neufeld makes a similar plea, saying that urotropin is almost invariably well borne. Suter² states that doses of from 15 to 25 grs. a day can be given for weeks in succession. Mogh³ certainly reports a burning and pricking sensation in the bladder, increased strangury, and appearance of red blood corpuscles in the urine following the use of the drug in cases of cystitis of gonorrhœal origin; but he employed much larger doses than those recommended above, giving 6 grams (or 92.6 grs.) a day. It seems worth while, therefore, to record two cases of hæmaturia following the use of 10 grs. of urotropin three times a day. The drug, though of undoubted value, is not quite so free from injurious effects as has been thought.