BACTERIOLOGICAL EXAMINATION OF TIDAL MUD AS AN INDEX OF POLLUTION OF THE RIVER.

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INSPECTION by a sanitary expert of all tidal rivers where oysters or other shell-fish are laid, or found, is of the highest importance, but it may frequently, with advantage, be supplemented by a bacteriological investigation.

Early in 1903, when my attention was drawn to the subject, I recognised that the bacteriological examination of the shell-fish alone might readily lead to misleading results owing to the probable want of uniformity of the pollution, if such had taken place, and so unequal distribution of the bacteria which are taken as the index of such pollution. At first I hoped that sampling the sea or tidal water over the beds would give more uniform results, but increased experience showed me that such samples were also liable to error and irregularity of results.

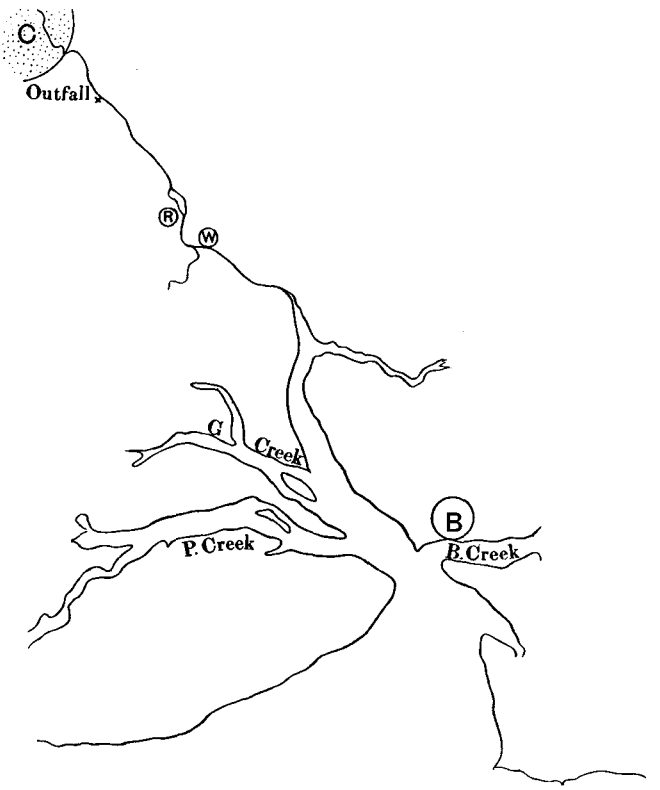
The Royal Commission on the treatment of disposal of sewage in their 4th Report drew attention to this matter. They state (p. 33) "it is clear that a single examination of chance samples of oysters might be most misleading as to the bacterial flora of the whole oyster-contents of a laying or pond," and remark that a similar caution is necessary in the case of waters. The bacterial content of the waters must obviously differ considerably according to the tide and especially whether ebb or flood water.

On theoretical grounds it seemed to me probable that the examination of samples of the river mud would yield results more uniform and more reliable; and it might well be possible from a bacteriological examination of a tidal mud to indicate how far such a position was suitable or unsuitable for shell-fish layings.

The following investigation deals with the results of a series of examinations of mud from a tidal river which has extensive oyster beds in creeks opening near the mouth of the river and beds also in the mouth of the river. About 8 miles from the mouth is a town of 40,000 inhabitants, which discharges its sewage into the river about a mile lower down. The sewer outfall is rather over 7 miles from the mouth of the river (see Map).

The only sources of pollution of this stretch of tidal river are as follows:

(1) The sewage of the town C. This is discharged into the river as above stated, but after treatment, while the plant is sufficiently large to enable the effluent to be discharged only on an ebb tide. The method of treatment consists of sedimentation in six large settling tanks followed by bacterial treatment in coke breeze and clinker beds (contact beds). The average amount of sewage dealt with is about 1,000,000



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gallons per day. The sludge from the sedimentation tanks does not run into the river, but is pressed into cakes and deposited on the land.

The effluent has always been clear when I have examined it and will keep indefinitely without putrefactive odours developing.

(2) From a village (R on map) about $1\frac{1}{2}$ miles below the sewer outfall of the town C small drains discharge into the river. Also $\frac{1}{4}$ mile below R but on the opposite side of the river, the drainage of another small place (W on map) with a population of 2,600, passes into the river.

Both W and R, however, have very few water-closets, and most of the privy contents finds its way directly on to land far away from the river.

(3) The drainage of the small town B (population about 4,600) is discharged into the mouth of the river by means of a single outfall, on a point 110 feet from the shore but above the low-water mark. The position was selected for the outfall after float experiments, which showed that the sewage effluent was conveyed out to sea by the currents and tides.

The sewage flows by gravitation into a precipitation tank, capacity 216,000 gallons, where it is treated with alumino-ferric. The sludge is disposed of on land, while the effluent is discharged into the sea during ebb-tide only, the valve being opened one hour after high water and closed one hour before low water. There is a storage tank capable of holding 100,000 gallons. This outfall is separated by the whole width of the estuary of the river from the creek P.

These are the only sources of sewage pollution. The oysters in creek P have been stated by Dr Bulstrode to be free from risk of pollution. Into creek P no drains discharge.

It must be clearly understood that the investigation here recorded was not undertaken to see if this particular river showed evidence of pollution or not.

The river is one the topographical characters of which have been repeatedly investigated by competent authorities and may be considered well known.

The main object was to see if the bacteriological examination of tidal mud from different parts would bear out the results obtained by careful topographical investigation, and how far such bacteriological mud examination may be taken as a reliable indication of the degree of pollution.

On topographical grounds the river may be divided into four parts.

I. *Obviously and markedly contaminated.* From the town of C to say a mile below the sewer outfall of this town.

II. *Considerable but less marked contamination.* From where I leaves off to, say, 1½ miles below township W.

III. *Slight or at least very diluted contamination.* The rest of the main channel of the river down to the mouth.

IV. *Free from any but extremely diluted sewage contamination.* The creeks P and G.

Here the water passing down the river does not at any time enter these channels for they are at the same time discharging their contents.

No drains run into them and the only sewage which can enter must be that which has passed out to sea, mixed with the many million gallons of pure sea water and then, if any, returned with the incoming tide.

Do the bacteriological results confirm these topographical considerations?

A number of samples were taken at different times, the results of which are set out in Tables I to IV.

The tables correspond to the topographical groups set out above.

Bacteriological methods of investigation.

In order to measure bacteriologically the degree of contamination it is necessary to have some reliable indication of contamination. The total number of bacteria present per gramme would probably be of but little service. A considerable experience with drinking-water, soils, etc. has convinced me of the especial value of *Bacillus coli* as such an indicator.

It is found in sewage and animal excretion in very large numbers, while there is no reliable evidence to show that it is found, or found in any but very small numbers, in soil, mud, or water which has not been contaminated by sewage or animal excretion.

The value of streptococci (as a class) has been less certainly and conclusively determined, but many workers (notably Houston) urge their great importance as a means of estimating excretal pollution.

The number of *B. coli* and of streptococci per gramme were taken therefore as the gauge of the amount of excretal contamination, the greater stress being laid upon the number of *Bacillus coli.* The number per gramme were determined in every case.

The samples were collected in sterile glass-stoppered bottles, and were in most cases examined within a few hours of collection; for a few the examination was made early next day. The state of the tide was recorded in every case, but it apparently made no difference to the bacterial content of the samples.

The same method of sampling and examination was used throughout so that the results should be strictly comparable.

1 grm. of the mud was carefully added by means of a sterile spatula to 9 c.c. of water in a 2 oz. wide mouth bottle (Kali pattern), fitted with a solid indiarubber cork, the whole bottle and contents being of course sterile. The indiarubber cork enabled the sample to be very thoroughly mixed. Then 1 c.c. of the diluted mud (labelled Dilution *A*), again well mixed *immediately* before the 1 c.c. was abstracted, was added to 99 c.c. of sterile water in a flask and labelled Dilution *B*. From *B* in the same way 1 c.c. was added, after intimate mixing to 99 c.c. of sterile water in a fresh flask (Dilution *C*). 0·1 and 1·0 c.c. of each of the dilutions were added to tubes of MacConkey's taurocholate medium and to neutral-red broth respectively. With muds where previous experience had shown the relative absence of *B. coli* and streptococci it was not necessary to also inoculate tubes from dilution *C* in every case. For *B. coli* MacConkey's medium was used. Neutral-red broth, though very valuable for *B. coli* detection in drinking-water, is in my experience much less suitable for sea waters and muds. The characteristic reaction is not always obtained, while other reducing organisms are sometimes present. The tube showing a positive result (gas and acid) with the greatest dilution was examined for *B. coli*, the organism being *in every case* isolated and its characters worked out. For the preliminary plating to isolate, Drigalski and Conradi's medium was used in the majority of cases. A positive result was not recorded unless a typical *B. coli* was isolated.

For streptococci the dilutions (0·1 and 1·0 c.c. of *A* and *B* and *C*) were added to tubes of neutral-red broth. These were incubated at 37° C. for 40–48 hrs. and then examined in hanging drops for streptococci. Frequently examinations after 24 hours' incubation were made in addition, but in every case hanging drop preparations were made after 40–48 hrs. I found the 40–48 hours' examination the best, as in no case did I find streptococci in 24 hrs. or 3 days in dilutions which did not show streptococci after 40–48 hrs., while the converse was not always the case. Several hanging drop preparations were always made from the dilution below the one giving positive results. No attempt was

made to isolate the streptococci, although I satisfied myself in every positive case that streptococci were present. These results must therefore be taken as indicating streptococci as a class, and do not deal with the different varieties present.

If *B. coli* or streptococci were found in 1 c.c. of Dilution *A* it was assumed that at least 10 per grm. of the mud were present and so for the other dilutions.

1 c.c. $A = 0{\cdot}1$ grm. mud; 0·1 c.c. dilution $A = 0{\cdot}01$ grm.;
1 c.c. dilution $B = 0{\cdot}001$ grm.; 0·1 c.c. dilution $B = 0{\cdot}0001$ grm.;
1 c.c. dilution $C = 0{\cdot}00001$ grm.; 0·1 c.c. dilution $C = 0{\cdot}000001$ grm.

Consideration of Tables I to IV.

Table I gives the results of samples taken from mud which on topographical grounds should be markedly polluted. Excluding No. 10 taken above the town C all the 10 samples show *B. coli* present in the proportion of at least 10,000 per gramme, while the number of streptococci present was never less than 100 per gramme.

In Table IV on the other hand the samples are from sources not subject to any but remote contamination. Here out of 8 samples *B. coli* numbered in one instance over 100 per gramme, but in the other samples 10 or less than 10 per gramme; while for streptococci the results showed 10 or less than 10 per gramme.

The contrast between these two sets of results is sharp and uniformly present.

The contamination of the samples in Table II would be only slightly less than in Table I, since the diminished contamination from the sewage of C owing to increased distance from the point of fouling would be partly compensated by the added pollution from the drainage of R and W. The bacteriological results of Table II bear this out, since they are but slightly better than those of Table I.

In the third group the self-purification factors and the much greater dilution of the sewage would cause the liability and possibilities of pollution to be vastly diminished; and the results of Table III show a marked improvement, *B. coli* being 100 per gramme (*i.e.* strictly speaking over 100 but less than 1000), and streptococci 10 per gramme.

A few samples were also taken from Creek B. These have a special interest of their own apart from the general interest of the inquiry.

The untreated sewage of the town B used to be discharged by three separate outlets on to the foreshore of the creek. Since 1899 the

sewage has been chemically treated and discharged at a single outfall at the junction of creek and river. It is discharged only at ebb tide and when it can be carried directly out to sea. In the creek there is also a considerable amount of traffic, boats being laid up in it during the winter.

The results of the five examinations (see Table V) are very unequal, as might be anticipated. Only near the ferry is there marked evidence of pollution. At the other parts of the creek a very considerable state of purity is evinced. Unfortunately no results are available for comparison with samples of mud taken before the sewage was diverted and treated.

The figures show that the purification effected has been most considerable, and, now that sewage no longer gains access to the creek, the mud compares very favourably with that from the main river.

These results I think clearly indicate that the examination of tidal mud gives valuable results, which closely accord with topographical data.

Before the full value of the bacteriological examination of tidal mud as a reliable measure of the actual amount of sewage or animal contamination can be gauged there are several obvious possibilities which must be considered.

In the first place do such mud examinations yield *uniform* results? The two factors which might be considered as possibly causing widely varying results are, in the first place, the influence of temperature or season, and secondly the irregularity caused by the contaminating material not being uniformly distributed but deposited locally and irregularly.

With regard to the first—the influence of temperature or season—samples were taken on purpose at very different times of the year. In the tables the date of collection is given in every case. A consideration of the tables does not show any marked variation due to season. Thus, for example, in Table II, samples 7 and 21 were taken from the same place as far as possible, one sample in February and one in August. Identical results were obtained both for *B. coli* and for streptococci.

With regard to irregularities of deposition of sewage-contaminated matters, on theoretical grounds this might be considered likely to occur, but in view of the constant agitation of the mud such irregularities of distribution would probably be quickly remedied. This point was however experimentally considered. Thus the three samples 23, 24, 25, were purposely taken as follows: No. 23 was collected about $\frac{3}{4}$ mile

below the sewage outfall of C from the left bank, tide coming in and about $2\frac{1}{2}$ hours after low water; No. 24 was collected on the same side but 100 yards above No. 23; and No. 25, about 100 yards above No. 24. All three samples were collected at the level of the water and immediately after one another. Identical results were obtained for both *B. coli* and streptococci, showing a remarkable uniformity of contamination so near the sewer outfall and over an area of about 200 yards.

Six other samples were specially collected from the creek P with this question in view. The results are shown in Table VI, but not in Tables I to IV. Here samples *a* and *d* were from the same place as regards distance from the mouth of the creek, but sample *a* almost at low water, and *d* at half-flood tide, in both cases at the level of the water. In the same way for samples *b* and *e*, and samples *c* and *f*, except that *b* and *e* were taken 100 yards further up the creek than *a* and *d*, and *c* and *f* 100 yards higher up still than *b* and *e*. It will be seen that almost identical results were obtained, five being identical and the sixth only differing in that *B. coli* were present in the proportion of 10 (less than 100) per gramme, while this organism was not found in $\frac{1}{10}$ gramme in the other samples.

I do not wish—and with so few samples it would not be justifiable to do so—to attach too much value to these figures, but as far as they go they certainly show marked uniformity, in striking contradistinction to results not infrequently obtained with the tidal sea water. Unreliability from local irregular contaminations seem to be, in the main, eliminated in mud samples. The constant stirring up of the mud would make for uniformity.

Another question which must be faced deals with the question of the time and age of the pollution. In other words, will tidal mud be a good index of present and recent contamination, or do the results merely show that contamination has taken place at some antecedent —perhaps long antecedent—period?

This consideration is obviously an important one, since if the detection of large numbers of *B. coli* and streptococci in a mud sample merely means that it has been polluted possibly at a very remote period, much of the significance of their detection is lost. For instance, if a mud is washed with a sea water comparatively pure, and containing let us suppose only *B. coli* and streptococci in 10 and 100 c.c. respectively, then these organisms will sink and contaminate the mud, and if they do not die out even a pure mud washed by a comparatively pure sea water, would in time yield high figures as regards *B. coli* and streptococci.

The results of Tables I to IV would show that this can scarcely be the case, while experiments of other workers have not demonstrated any multiplication of *B. coli* in mud. I am not acquainted with any direct experiments in this connection with regard to streptococci in tidal mud. I have, however, made a number of direct experiments, since on other grounds as well—notably the question of the sensitiveness of mud examinations as indicators of degree of contamination—the matter merits further consideration.

My results may be recorded under two divisions:

A. Experiments with moist muds kept in stoppered bottles.

B. Experiments with muds kept as nearly as possible under natural conditions.

Series A. Four muds were in this way kept in stoppered bottles and reexamined after an interval of a week or more.

The results are shown in Table VII.

This table shows that with all four muds the number of streptococci per gramme rapidly diminished, and that the number of *B. coli* also decreased.

In no case was there any observable increase on keeping, of either *B. coli* or streptococci.

These muds however received no sea water and could not be considered as kept under natural conditions.

Series B. In this series several samples of mud were kept in open tanks in a shed. Several pounds of mud were used for each experiment, and each mud sample received twice a week $1\frac{1}{2}$ gallons or more of fresh sea water, the mud and water being thoroughly mixed after each addition. The mixture was then allowed to stand until the next sample of sea water was received. The supernatant sea water was then carefully siphoned off, and the fresh sea water added and thoroughly mixed. The tanks were kept in a shed, the temperature of the water and of the air being carefully recorded each morning.

Here the conditions approximated to a certain extent to those met with in nature.

In order to obviate the risk of added *B. coli* and streptococci in the sea water, for most of the experiments of this series, the sea water was partially sterilized in the steam sterilizer and heated up until steam was evolved. The water was then removed and rapidly cooled by standing the vessels in cold water.

Tank I. Mud collected Aug. 30, 1904, from the river about $\frac{1}{4}$ mile below the sewage outfall pipe. Put into the tank, and sea water added

Aug. 31st. The sea water was added twice a week and varied from $1\frac{1}{2}$ to $2\frac{1}{2}$ gallons at a time. In every case it was partially sterilized as above. The examinations were made in the same way as for samples collected under natural conditions. The results of the examinations are given in Table IX.

Tank II. Mud collected from the river about 80 yards below the sewage outfall. Collected Sept. 12th, 1904, and put into the tank and started within a few hours of collection. Experiment exactly as for Tank I, except that at first the sea water was added unheated or untreated in any way. On and from Oct. 25th the sea water was partially sterilized exactly as for Tank I. The results of the examinations are given in Table X.

Tank III. Mud collected from the river Oct. 20th about $\frac{1}{2}$ mile above the sewage works. Put into the tank and sea water added next day. Partially sterilized sea water added biweekly exactly as for Tank I. The results of the examinations are given in Table XI.

Temperature during the tank and other experiments. The temperature of the air of the shed and of the water was recorded every morning at 9.0 a.m. throughout the experiments, *i.e.* from Aug. 31st to Dec. 14th, 1904. I do not think it necessary to burden the paper with these temperatures in detail. The following data will give a general idea of the temperature variations. Air temperatures from Aug. 31st to Sept. 9th, 13°–15° C., Sept. 10th to 12th 11°–12° C., Sept. 13th to 19th 13°–15° C., Sept. 19th to Oct. 6th 11°–13° C., Oct. 7th to 16th 5°–12° C., Oct. 19th to 24th 12°–15° C., Oct. 25th to Nov. 11th 9°–11° C., Nov. 12th to 17th 5°–8° C., Nov. 18th to 21st 0°–4° C., Nov. 22nd to 29th 7°–9° C., Nov. 30th to Dec. 6th 3°–6° C., Dec. 7th to 13th 0°–4° C.

These are air temperatures, the temperature of the tank water was usually 1–2 degrees lower.

As, for part of the experiments, fresh unheated sea water was added to the tanks it is of interest to have some idea of the bacterial content of the sea water, which was obtained by rail from the G. E. Railway. Six bacteriological examinations were made. The following is a summary of the results as regards the numerical presence of *B. coli* and streptococci.

Examined Sept. 13th. *B. coli* present in 10 c.c. not in $\frac{1}{10}$, $\frac{1}{2}$, 2 c.c.; streptococci not in 12 c.c.; larger amounts not examined, *B. coli* isolated quite typical.

Examined Sept. 27th. *B. coli* absent in $\frac{1}{10}$, $\frac{1}{2}$, 2, 10 c.c.; no streptococci in 50 c.c.

Examined Oct. 4th. *B. coli* in 2, 10, 40 c.c., smaller amounts not examined. *B. coli* isolated, typical, except that no milk coagulation (3 weeks' incubation: repeated same result). Streptococci in 10 and 40 c.c. smaller amounts not examined.

Examined Oct. 14th. *B. coli* absent in $\frac{1}{10}$, $\frac{1}{2}$, 2, 10 c.c. streptococci present in 10 c.c., smaller amounts not examined.

Examined Oct. 21st. *B. coli* present in $\frac{1}{2}$, 2, 10 c.c. Quite typical characters. Streptococci in 10 c.c., less not examined.

Examined Dec. 7th. *B. coli* and also streptococci absent in 2 and 10 c.c., larger amounts not examined.

It will be seen that the bacterial content, as regards *B. coli* and streptococci, of the sea water supplied, varied very considerably.

Examinations of Tables IX, X, and XI, show on the whole very similar results for all three tank experiments.

All three gave identical results when started, *i.e.* *B. coli* greater than 100,000 but less than 1,000,000 per gramme, and streptococci greater than 1,000 but less than 10,000 per gramme of mud.

A gradual, but not perfectly regular, decline takes place week by week in the number of both *B. coli* and streptococci.

The decline for Tank II which up to Oct. 25th received unheated sea water, containing living *B. coli* and streptococci often, is naturally slower than for Tank I.

The results obtained show that streptococci usually diminish and die out much more rapidly than *B. coli* and are thus indicators of more recent contamination of tidal mud. Tank III results indicate however that some forms of streptococci are apparently very resistant. From my whole series of experiments I should however conclude that these are comparatively rare in tidal mud.

B. coli diminish in numbers, at first fairly rapidly, but when the number is reduced to 100–1000 per gramme, the decline is comparatively slow. Further, although with time, markedly greater diminution does take place, yet even in muds kept under these, as far as possible, natural conditions the *B. coli* do not quite disappear, but are found in the proportion of at least 1 per gramme, and this after the lapse of three months from the initial pollution.

These experiments emphasise the great importance of the *enumeration* of *B. coli* in estimating pollution. The mere detection of *B. coli* is valueless. Here are muds in which after the lapse of three months (during which two at least (Tanks I and III) have received no fresh

B. coli), this organism can still be found, if one gramme of the mud be examined.

On the other hand when the *quantitative* aspect is considered, it is seen that there is a vast difference between the freshly contaminated mud with more than 100,000 *B. coli* per gramme, and the mud not polluted for three months, with but 1–10 *B. coli* per gramme.

The tank experiments, in my opinion, confirm the view that tidal mud is a good index of present and recent pollution of the river when the results are properly interpreted.

They indicate that the examination of tidal mud will not only show that pollution has taken place, but will give some indication as to the time and amount of such pollution.

They further show that the presence of *B. coli* in 0·1 gramme of mud cannot be considered as indicating recent contamination, unless by material itself very slightly polluted.

For Tank I, 6 weeks after the experiment was started, which may be considered as 6 weeks after the pollution of the mud with sewage, *B. coli* were still found in 0·1 grammes: for Tank II this organism was present in the same amount after 9 weeks: while for Tank III 5½ weeks after the pollution *B. coli* were still present in 0·01 gramme.

No doubt however under quite natural conditions, and with a washing of the mud at each tide with a pure sea water, a more uniform and rapid decline in the number of both *B. coli* and streptococci would have taken place.

It is of interest to notice that these laboratory results are quite in accord with the samples taken from the river, as given in Table IV. The results given in Table IV show that these muds which on topographical grounds are certainly free from pollution, except of remote and extremely diluted kind, almost constantly contained *B. coli* to the number of at least 10 per gramme, but only in one case to the number of at least 100 per gramme.

The question of specific pollution with the typhoid bacillus of tidal water or tidal mud must also be directly considered.

Are we in a position to say from the results of the examination of tidal mud, *e.g.* from an enumeration of *B. coli* and streptococci, that the typhoid bacillus is certainly absent from such tidal mud?

In the first place it will I think be conceded that if typhoid bacilli do gain access to a tidal river they will almost certainly be mixed with a preponderating number of *B. coli* and probably also with streptococci more numerous than themselves. If the pollution is by *urine*, infected

with the typhoid bacillus, this may not be the case, but such an infection must be a very exceptional one, and in general, specific pollution with typhoid bacilli means also a concurrent pollution with vast numbers of *B. coli* and streptococci. In other words, the muds of Tanks of I, II, and III might readily be muds also specifically polluted with the typhoid bacillus.

How long is this latter organism likely to survive in such muds, or put in another way, what numbers of *B. coli* and streptococci are sufficient to enable us to say with certainty, or at least practical certainty, that such a mud, and so presumably any shell-fish in it, is free from typhoid bacilli?

It is usually freely assumed that *B. typhosus* will die out more rapidly than *B. coli*, and probably this is true, but no exact experiments have been made, to my knowledge, with tidal mud.

I have therefore made these questions the subject of a number of direct experiments.

Group I. B. typhosus in sterile moist tidal mud.

If the typhoid bacillus died out rapidly in sterile mud the question would be readily answered whether results with regard to *B. coli* and streptococci are sufficient to indicate absence of the typhoid bacillus.

Two experiments were undertaken to estimate the vitality of *B. typhosus* in sterile mud. In most of the experiments published in regard to the vitality of this organism in sterile fluids, only the presence or absence of the organism has been usually determined. Here quantitative estimations were made so that the rate of increase or decrease could also be studied.

Exp. 1. Mud collected from the river and from a source similar to No. 22, Table II: Mixed with a little sea-water and sterilized in a flask with cotton-wool stopper for 45 minutes at 115° C. in the autoclave. The mud was of such consistency that after mixing well, it could be drawn up into an ordinary sterile graduated 1 c.c. pipette. One loopful of a two-day broth culture of *B. typhosus* (isolated some months previously from a case of typhoid fever) added. For this experiment the flask was kept stoppered with an indiarubber plug and standing in a basin of water in an outside shed where the other experiments were carried out; the temperature of shed and water is given above for all these experiments.

The numbers of *B. typhosus* present were estimated at once and then at intervals as indicated in Table XII.

1 c.c. of the liquid was added to 9 c.c. sterile water = dilution *A*.
1 c.c. dilution *A* added to 99 c.c. = dilution *B*.
1 c.c. dilution *B* added to 99 c.c. = dilution *C* &c.

1·0 and 0·1 c.c. of each dilution added to broth tubes which were subsequently examined for *B. typhosus*. The organism isolated in the greatest dilution for the last examination made, was fully worked out and identified: the others only partially.

Exp. 2. Exactly similar to Exp. 1, except that the mud was rather less liquid and the enumeration was made by adding two platinum loopfuls of mud each time to 9 c.c. sterile water = dilution *A*. Dilutions *B* and *C*, as for Exp. 1. Both experiments started Sept. 2nd, 1904. For the results of the examinations see Table XII *a*.

The characters of the typhoid bacilli isolated after 50 days (Exp. 1) and 56 days (Exp. 2) respectively were worked out and found to be quite unaltered.

These two experiments demonstrate that the typhoid bacillus will readily live in sterile moist mud. They further show that under the conditions of experiment an increase in numbers took place, followed by a diminution; but at the end of 7—8 weeks there was either about the same number or fewer bacilli present than when the experiment was started. They also show that the characters of the typhoid bacillus were not affected by prolonged sojourning in sterile mud. These results although of interest in themselves do not answer the essential question propounded.

Group II. B. typhosus in polluted tidal mud (not sterilized).

This group comprises four separate experiments. For all four experiments large numbers of typhoid bacilli were added to fresh highly polluted tidal river mud in sterile flasks of 300—400 c.c. capacity. The flask was filled with fresh sea water and the mud and sea water thoroughly mixed. Fresh sea water was added twice a week, the procedure being in each case to pour off the stale sea water, take samples of the mud if required, add the fresh sea water, and mix up thoroughly. The flasks were plugged loosely with cotton-wool and kept in the outside shed. For temperatures of the shed see above.

To demonstrate the typhoid bacillus several separate platinum loopfuls of the mud were brushed over series of Drigalski and Conradi plates. All typhoid like colonies were subcultivated into broth and incubated at 37°C. These broth cultivations were examined next day and all those which showed uniform turbidity of the broth and in hanging drop, a possible morphology, and considerable motility were further examined. The others were at once excluded.

The further steps employed were to grow these organisms in lactose peptone water (in Durham's tubes) for acid and gas production, in litmus milk for at least 2 weeks and to test the 24 hours' broth culture in 1 : 1000 dilution with a powerful antityphoid serum (time allowed 2 hours) which readily agglutinated the race of *B. typhosus* used for these experiments in this and higher dilutions. The further tests employed for identification were growth in peptone water for indol production, in

glucose neutral-red agar shake cultures, and on gelatin slope cultures for slow growth, typical appearance and non-liquefaction. The typhoid bacilli last isolated were further stained by Gram's method, grown in mannite peptone water and their gelatin surface colonies examined, while the reactions with the antityphoid serum were repeated.

The organism isolated had to conform in all its characters to that of the typhoid bacillus used for the experiments, before it was accepted as that organism.

Exp. 1. Started Sept. 16th, 1904. 2 c.c. of emulsion in sea water of *B. typhosus* agar slope culture, added.

1*st examination* Sept. 16th. Typhoid bacilli colonies abundant in one loopful of the mud, brushed over a Drigalski and Conradi plate.

2*nd examination* Sept. 30th. *B. typhosus* fairly readily isolated.

3*rd* „ Oct. 7th. *B. typhosus* not isolated.

4*th* „ Oct. 11th. „ „ „

Result. Typhoid bacilli fairly easily isolated after two weeks in the mud, but could not be found after three weeks.

Exp. 2. Started Sept. 16th, 1904. 1·5 c.c. of emulsion in sea water of *B. typhosus* agar slope culture, added.

1*st examination* Sept. 16th. *B. typhosus* easily isolated from 1 loopful of mud.

2*nd* „ Sept. 22nd. „ „ „ „ 1 „ „

3*rd* „ Sept. 30th. *B. typhosus* fairly readily isolated.

4*th* „ Oct. 7th. *B. typhosus* not isolated.

5*th* „ Oct. 14th. „ „ „

Result. Identical with Experiment 1.

Exp. 3. Started Oct. 21st, 1904. Here part of the same mud as that of Tank III was used.

For this experiment an attempt was made not merely to see if the typhoid bacillus was present but to as far as possible estimate its numerical presence. At the same time the number of *B. coli* and streptococci were also estimated every week exactly in the same way as for the tank experiments. The results of the *B. coli* and streptococci enumerations are given in Table XIII.

To estimate the number of *B. typhosus* not only were loopfuls of the mud brushed over Drigalski and Conradi plates, but the MacConkey tubes each containing 0·1, 0·01, etc. grm. of mud were also brushed over similar plates after 1–2 days' incubation at 37° C. A considerable part of an agar slope culture emulsified in fresh sea water was added to the mud (about 100 grms.) so that the infection was a massive one.

1*st examination* Oct. 21st. Here the colonies from one loopful of mud appeared all blue, and supposing they were all *B. typhosus* I only subcultivated one. On working out it was found to be not *B. typhosus*, but an organism closely simulating it. Its most characteristic feature was the marked alkalinity it produced in litmus milk after 8–9 days' growth, for the first few days acid being produced. It did not ferment glucose like *B. enteritidis* however. *B. typhosus* was therefore not isolated.

2*nd examination* Oct. 29th. *B. typhosus* isolated from 0·00001 grm. as well as from one loopful of mud.

3*rd examination* Nov. 5th. *B. typhosus* isolated from 0·00001 grm. of mud.

4*th examination* Nov. 11th. *B. typhosus* isolated from 1 loopful of mud, but not found in any of the dilutions.

5*th examination* Nov. 18th. *B. typhosus* isolated from 1 loopful of mud but not found in any of the dilutions.

6*th examination* Nov. 25th (after 35 days). Quite similar to Nov 18th, *i.e.* isolated from 1 loopful of mud.

7*th examination* Dec. 2nd. *B. typhosus* not isolated.

8*th* „ Dec. 9th. „ „ „

Result. In this experiment the typhoid bacillus was isolated after 35 days in mud, but could not be found a week later.

When the typhoid bacillus was found *B. coli* were still present in 0·01 grm. (see Table XIII), but streptococci could not be found in 0·1 grm.

The infection with typhoid bacilli was a very gross one however, and even after 2 weeks in the mud the bacillus was isolated in 0·00001 grm. of mud: *i.e.* there were then presumably at least 100,000 typhoid bacilli per gramme of mud, numbers at least equal to the number of *B. coli* present.

Exp. 4. Started Oct. 21st, 1904. The same mud as Exp. 3, and inoculated with approximately an equal amount of agar emulsion. The detection of the bacillus alone was however attempted.

1*st examination* Oct. 29th (after 8 days). *B. typhosus* easily isolated from 1 loop of mud.

2*nd examination* Nov. 5th (after 15 days). *B. typhosus* fairly easily isolated.

3*rd* „ Nov. 11th (after 21 days). *B. typhosus* not isolated.

4*th* „ Nov. 15th. *B. typhosus* not isolated.

5*th* „ Nov. 16th. „ „ „

Result. *B. typhosus* fairly easily isolated after 15 days, but could not be found after a longer interval.

Owing to the enormous difficulties inherent to the isolation of the typhoid bacillus from bacteriologically complex substances such as highly polluted tidal mud, it would be rash to draw sweeping deductions from negative results, but from the above it seems justifiable to infer that typhoid bacilli can survive in polluted muds for at least 2 weeks, and this fairly readily, but that after about 2 weeks they very rapidly decrease, although they may, and probably do, persist under favourable conditions for some time longer but in vastly diminished numbers. Experiment (3) seems to definitely show that they may survive for at least 5 weeks.

The fact that the typhoid bacilli may have been present in the mud but not isolated owing to the inherent difficulties of such isolation is probably entirely compensated for by the very massive infection practised, and when the rate of decrease of *B. coli* and streptococci in the

tank muds is considered it seems to me justifiable to assume that if *B. coli* are present in less than 0·01 gramme and streptococci only found in 0·1 gramme or less, the risk of the typhoid bacillus being present, and consequently of specific oyster pollution, can be considered a negligible one.

Group III. B. typhosus + B. coli in sterile mud.

In view of the difficulty of, with certainty, isolating the typhoid bacillus from polluted tidal mud, it occurred to me that if it could be shown that *B. coli* readily killed out *B. typhosus* in sterile mud, to which both had been added, then the problem of the possibility of excluding the presence of the typhoid bacillus from an estimation of the number of *B. coli*, might be answered in another way.

Two separate experiments were carried out from this point of view.

Exp. 1. Polluted tidal mud sterilized in bottle with cotton-wool plug, Oct. 27th. 1 loopful added of an agar slope emulsion in 5 c.c. of sterile water of a typical *B. coli* isolated from mud. Also 1 c.c. of a sterile water emulsion of *B. typhosus* from agar slope. The mud which was quite moist was well mixed.

Examinations made by weighing 1 grm. into 9 c.c. of sterile water = dilution *A*. Dilutions *B*, *C*, *D*, etc. from this as above described.

The number of *B. coli* was determined by adding 1·0 and 0·1 c.c. of each dilution to MacConkey tubes for gas and acid.

The number of *B. typhosus* was ascertained by plating some of these dilutions and also by brushing directly 1 drop (approximately $\frac{1}{30}$ c.c.) of each dilution over a plate, Drigalski and Conradi plates being used throughout. Care was taken to show that only these two organisms were present throughout the experiment.

The results are shown in Table XIV.

Exp. 2. Started Oct. 29th. Rather over 60 grms. of moist mud sterilized in flask with cotton-wool plug.

1 c.c. of *B. typhosus* agar emulsion in sterile tap-water added. The *B. coli* was not added until Nov. 2nd, to give the typhoid bacilli a start, when 0·1 c.c. of a gelatin slope emulsion in tap-water was added. Otherwise the experiment was quite similar to Exp. 1. Both flasks kept in the same shed as the other muds. The results are shown in Table XV.

In both experiments the typhoid bacillus was originally present in larger numbers than the colon bacillus.

Both experiments show that the two bacilli can maintain themselves side by side, and in considerable numbers, in tidal mud for some time—apparently 3–4 weeks under the conditions of the experiments. After that period the typhoid bacilli find the conditions unsuitable and

apparently rapidly die out, or at least are present in vastly diminished numbers. On the other hand the *B. coli* were in both instances present in larger numbers at the end of the experiment than when the experiments were started.

Comparing these results with those obtained with *B. typhosus* alone in sterile mud, it is evident that the colon bacillus exerts after a while a prejudicial influence on the growth of the typhoid bacillus in tidal mud. This is what might be expected, although I was not prepared to find that it was not exerted until after several weeks.

Group IV. Two further experiments were carried out—one with streptococci, the other with *B. coli* in sterile mud.

Exp. 1. A streptococcus isolated from mud was mixed with sterile mud and the number estimated every week. Flask kept in shed. Started Oct. 25th. Streptococci when the experiment was started were more numerous than 10, but less than 100 per grm.

Nov. 1st (after 1 week). Streptococci present in 0·01 grm. (*i.e.* more numerous than 100 per grm.) but greater dilutions not examined.

Nov. 7th (after 13 days). More numerous than 100, less than 1000 per grm.

Nov. 17th (after 23 days). „ „ „ „ „ „ „ „ „

Nov. 29th (after 35 days). Not found in 0·1 grm. or less.

Dec. 5th (after 41 days). Absent in 1 grm. or less.

In this experiment the streptococcus maintained itself, with a slight increase, up to 3 weeks, but then, or soon after, rapidly died out.

Exp. 2. A typical *B. coli* isolated from mud mixed with sterile mud in flask, and number present estimated after definite intervals. Started Oct. 25th, 1904. *B. coli* numbered more than 10,000, less than 100,000 per grm.

Nov. 8th (after 14 days). *B. coli* numbered more than 10,000, less than 100,000 per grm.

Nov. 23rd (after 29 days). *B. coli* numbered more than 1000, less than 10,000 per grm.

Nov. 29th (after 35 days). „ „ „ „ 10 „ „ 100 „

Dec. 6th (after 42 days). „ „ „ „ 100 „ „ 1000 „

In this particular experiment a distinct diminution in numbers was obtained, but the bacillus could maintain itself for at least 6 weeks, although after 4 weeks a marked diminution took place.

It may further be mentioned that the *B. coli* isolated from these sterile muds were fully worked out on several occasions after being in sterile mud for 5 or 6 weeks, and were found to have retained their characters unaltered.

None of my experiments bear out the idea that *B. coli* in mud, sterilized or unsterilized, alter some of their characters.

Conclusions.

(1) That mud samples yield more reliable bacteriological evidence of the degree of contamination of a tidal river than either water or oyster samples.

(2) Oyster and water samples only indicate immediate and actually present pollution. Mud samples show evidences of past contamination for at least several weeks, and almost certainly for all the time that specific (typhoid bacilli) contamination is possible.

(3) Muds which show high relative purity are safe for oysters.

(4) Standards of number of *B. coli*, streptococci, etc. if broad, can be set up and will serve as a useful classification of the degrees of purity of a tidal river, and will aid inspection and possibly be largely able to take its place.

I am not prepared at present to give a numerical standard, or from the above experiments to affirm that the same standard is applicable to all rivers.

(5) No evidence was obtained that either *B. typhosus* or *B. coli* alter their characters in tidal mud.

(6) Typhoid bacilli can survive fairly readily for 2 weeks in tidal mud, but after that period their numbers, as a rule, rapidly decline.

TABLE I.

No. in Series	Date of Collection	Source of the sample	Ascertained presence of *B. coli* in amounts of wet mud indicated (Grammes)						Do. for Streptococci (Grammes)					Estimated No. per grm.	
			0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Streptococci
2	Jan. 22, '04	Just below the town C and above the sewage outfall	+	+	+	+	+	+	+	+	+	+	−	over 1,000,000	10,000
10	Feb. 24, '04	River above the town C	+	+	−	−	−	−	+	+	+	−	−	100	1,000
14	March 15, '04	About 20 yards below No. 2	+	+	+	+	+	−	+	+	+	−	−	100,000	1,000
15	,, ,,	100 yards above sewage outfall	+	+	+	+	−	−	+	+	+	+	−	10,000	10,000
16	,, ,,	100 yards below sewage outfall. Left bank	+	+	+	+	−	−	+	+	+	−	−	,,	1,000
17	,, ,,	100 yards below sewage outfall. Right bank (same side as outfall)	+	+	+	+	−	−	+	+	+	+	−	,,	10,000
23	Aug. 30, '04	About ¾ mile below sewage outfall. Left bank	+	+	+	+	−	−	+	+	−	−	−	,,	100
24	,, ,,	About 100 yards above No. 23	+	+	+	+	−	−	+	+	−	−	−	,,	100
25	,, ,,	About 100 yards above No. 24	+	+	+	+	−	−	+	+	−	−	−	,,	100
26	,, ,,	¼ mile below sewage outfall. Left bank	+	+	+	+	+	−	+	+	+	−	−	100,000	1,000
27	Sept. 12, '04	100 yards below sewage outfall. Right bank	+	+	+	+	+	−	+	+	+	−	−	,,	1,000

TABLE II.

7	Feb. 2, '04	Main channel ¼ mile below township W	+	+	+	−	−	−	+	+	−	−	−	1,000	100
18	June 13, '04	40 yards below where largest W drains enter the river	+	+	+	+	−	−	+	+	+	−	−	10,000	1,000
20	Aug. 30, '04	About ⅓ mile below township W	+	+	+	+	−	−	+	+	−	−	−	,,	100
21	,, ,,	½ mile below township W	+	+	+	−	−	−	+	+	−	−	−	1,000	100
22	,, ,,	Between R and W	+	+	+	+	−	−	+	−	−	−	−	10,000	10

TABLE III.

No. in Series	Date of Collection	Source of the sample	Presence of *B. coli* in amounts indicated (Grammes)						Do. for Streptococci (Grammes)					Estimated No. per grm.	
			0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Streptococci
5	Feb. 2, '04	Main channel. ½ mile above creek G. Right bank	+	+	–	–	–	–	+	–	–	–	–	100	10
6	,, ,,	Main channel. Just before creek B. Left bank	+	+	–	–	–	–	+	–	–	–	–	100	10
13	Feb. 26, '04	Main channel between creeks G and P	+	+	–	–	–	–	+	–	–	–	–	100	10
28	Oct. 10, '04	Main channel. Left bank. Just above mouth of creek B. About 200 yards above sewage outfall of town B	+	+	–	–	–	–	–	–	–	–	–	100	less than 10
29	,, ,,	Main channel. Left bank. 200 yards above last sample	+	+	–	–	–	–	–	–	–	–	–	100	,,

TABLE IV.

1	Dec. 31, '03	Creek G near mouth	+	–	–	–	–	–	–	–	–			10	less than 10
3	Feb. 2, '04	Creek P, ⅓ mile up from mouth	+	–	–	–			+	–	–			10	10
4	,, ,,	Creek P, 50 yards from mouth	+	–	–	–			–	–	–			10	less than 10
8	,, ,,	Creek P, ⅓ mile up from mouth	+	–	–	–			–	–	–			10	,,
9	,, ,,	Creek G, about 200 yards up	+	+	–	–	–	–	+	–	–			100	10
11	Feb. 26, '04	Creek P, ¾ mile up	+	–	–	–			+	–	–			10	10
12	,, ,,	Creek G, ¾ mile up	+	–	–	–			–	–	–			10	less than 10
19	June 29, '04	Creek P, rather less than ¼ mile from mouth	–	–	–	–			–	–	–			less than 10	less than 10

TABLE V.

No. in Series	Date of Collection	Source of the sample	Presence of *B. coli* in amounts indicated (Grammes)						Do. for Streptococci (Grammes)					Estimated No. per grm.	
			0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Strepto-cocci
30	Oct. 10, '04	Creek B. About ½ mile up the creek from the ferry. Right bank	+	–	–	–	–	–	–	–	–	–	–	10	less than 10
31	,, ,,	Creek B. About 250 yards nearer the ferry than last sample. Right bank	–	–	–	–	–	–	–	–	–	–	–	less than 10	,,
32	,, ,,	Creek B. About 200 yards nearer mouth than last sample. Right bank	+	–	–	–	–	–	–	+	–	–	–	10	?
33	,, ,,	Creek B. About 50 yards up the creek from the ferry. Right bank	+	+	+	+	–	–	+	+	–	–	–	10,000	100
34	,, ,,	Creek B. Left bank. Near mouth and seaward side of the ferry.	+	–	–	–	–	–	+	+	–	–	–	10	100

TABLE VI.

a	June 29, '04	Creek P. ⅓ mile up from mouth. Nearly low water	–	–	–	–			–	–	–			less than 10	less than 10
b	,, ,,	Creek P. 100 yards higher up than *a*. Collected at same time	+	–	–	–			–	–	–			10	,,
c	,, ,,	Creek P. 100 yards higher up than *b*. Collected at same time	–	–	–	–			–	–	–			less than 10	,,
d	,, ,,	Creek P. Same place as *a* except that at ½ flood tide, *i.e.* higher level	–	–	–	–			–	–	–			,,	,,
e	,, ,,	Creek P. Same as *b*, but at ½ flood tide	–		–	–			–	–	–			,,	,,
f	,, ,,	Creek P. Same as *c*, but at ½ flood tide	–	–	–	–			–	–	–			,,	,,

TABLE VIII. *Characters of B. coli isolated.*

Reference number	Source	Liquefaction of Gelatine	Gelatine Slope		Glucose Fermentation	Lactose Fermentation	Neutral red Reaction	Litmus Milk		Indol production	Morphology	Motility	Saccharose Fermentation	
			2 days	2 weeks				Permanent acidity 2 weeks	Coagulation					
1	Mud 1	–	Bluish translucent growth	unchanged	+	+	+	+	+ (3 days)	+	Short b. rounded ends	+		
2	,, 2	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
3	,, 3	–	Transl. growth, but whiter than quite typical	,,	+	+	+	+	+ (24 hrs.)	+	,,	–	+	
4	,, 4	–	Bl. transl. growth	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
5	,, 5	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–	–	
6	,, 6	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	+	–	
7	,, 7 (0·001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–	–	
8	,, 8 (0·1 grm.)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+	–	
9	,, 9 (0·01 ,,)	–	,,	,,	+	+	–	+	+ (24 hrs.)	+	,,	–	–	
10	,, 10 (0·01 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	+	–	
11	,, 11 (0·1 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
12	,, 12 (0·1 ,,)	–	,,	,,	+	+	+	+	+ (6 days)	+	,,	+		
13	,, 13 (0·01 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–	+	
14	,, 14 (·00001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
15	,, 15 (·0001 grm.)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–	–	
16	,, 16 (0·0001 grm.)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
17	,, 17 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
18	,, 18 (,, ,,)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	+	–	
19	,, 20 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
20	,, 21 (·001 grm.)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
21	,, 22 (·0001 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	–		
22	,, 23 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	–		
23	,, 24 (,, ,,)	–	,,	,,	+	+	+	+	+ (6 days)	–	,,	+		
24	,, 25 (,, ,,)	–	,,	,,	+	+	+	+	+ (,,)	+	,,	+	+	Ferments glucose neutral red shake but slightly at first, and with no neutral red reaction.
25	,, 26 (·00001 grm.)	–	,,	,,	–	+	–	+	+ (2 days)	+	,,	–	+	
26	,, 27 (,, ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
27	,, 28 (·01 ,,)	–	,,	,,	+	+	+	+	+ (3 days)	+	,,	+		
28	,, 29 (,, ,,)	–	,,	,,	+	+	+	+	+ (2 days)	+	,,	–		
29	,, 30 (·1 ,,)	–	,,	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
30	,, 32 (0·1 ,,)	–	Less translucent & whiter than typ. not crinkled	,,	+	+	+	+	+ (2 days)	+	,,	–		
31	,, 33 (0·0001 grm.)	–	Bl. transl. growth	,,	+	+	+	+	+ (24 hrs.)	+	,,	–		
32	,, 34 (0·1 grm.)		,,	,,	+	+	+	+	+ (2 days)	+	,,	–		

34	„ 13 (0·1 grm.)	–	growth	changed	+	+	+	+	+ („)	–	rounded ends	+		later. Indol repeated after 1 week. Marked indol present.
35	„ 10 (0·01 „)	–	„	„	+	+	+	+	+ („)	+	„	+		
36	„ 15 („ „)	–	„	„	+	+	+	+	+ (3 days)	+	„	–		
37	„ 15 („ „)	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
39	Tank I. mud	–	„	„	+	+ slow	partial	+	+ (3 days)	+	„	+	+	Neutral red agar shake repeated several times with same result.
40	„ „	–	„	„	+	+	+	+	+ (2 „)	+	„	+		
41	„ „	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–	+	
42	„ „	–	„	„	+	+	+	+	+ („)	+	„	–	–	
43	„ „	–	„	„	+	+	+	+	+ („)	–	„	–		Indol test repeated. Same result.
44	„ „	–	„	„	+	+	+	+	+ („)	+	„	–		
45	„ „	–	Translucent growth thicker & moister than typical form	„	+	+	+ delayed	+	+ (2 days)	+	„	–	–	No starch fermentation.
46	„ „	–	Bl. transl. growth	„	+	+	+	+	+ („)	+				
47	„ „	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
48	„ „	–	„	„	+	+	+	+	+ (6 days)	+	„	–		
49	„ „	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
50	Tank II. mud	–	„	„	+	+	+	+	+ („)	+	„	–		
51	„ „	–	Thick white growth	„	+	+ +	+	+	+ („)	–	„	–	+ +	Ferments starch slightly. Also raffinose but not dulcite. *Not B. coli.*
52	„ „	–	Bl. transl. growth	„	+	+	+	+	+ (2 days)	+	„	–		
53	„ „	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
54	„ „	–	„	„	+	+	+	+	+ (4 days)	+	„	–		
55	„ „	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
56	„ „	–	„	„	+	+	+	+	+ (2 days)	+	„	+		
57	„ „	–	„	„	+	+	+	+	+ („)	+	„	–		
58	„ „	–	Whiter and thicker than typical	„	+	+	partial only	+	+ (24 hrs.)	+	„	–		No starch fermentation.
59	„ „	–	Bl. transl. growth	„	+	+	+	+	+ (2 days)	+	„	–		
60	„ „	–	„	„	+	+	+	+	+ („)	+	„	–		
62	Tank III. mud	–	„	„	+	+	+	+	+ (24 hrs.)	+	„	–		
63	„ „	–	„	„	+	+	+	+	+ (2 days)	+	„	–		
64	„ „	–	„	„	+	+	+	+	+ („)	+				
65	„ „	–	„	„	+	+	partial	+	+ (24 hrs.)	–	„	–		Repeated 1 month later. Indol produced.
66	„ „	–	„	„	+	+	+	+	+ („)	+	„	–		
67	„ „	–	„	„	+	+	+	+	+ („)		„	–		
68	Group II. Exp. (3)	–	„	„	+	+	+	+	+ (2 days)	+	„	+		
69	„ „	–	„	„	+	+	+	+	+ („)	+	„	–		
70	„ „	–	„	„	+	+	+	+	+ (3 „)	+	„	–		
71	„ „	–	„	„	+	+	+ delayed	+	+ (24 hrs.)	+	„	–		
72	„ „	–	Whiter and moister than typical	„	+	+	–	+	+ („)	–	„	–	–	Neutral red positive reaction given after one week, after being kept in the laboratory for 2 weeks.
73	„ „	–	Bl. transl. growth	„	+	+	–	+	+ („)	+				
74	„ „	–	„	„	+	+	+	+	+ (2 days)		„	+		

TABLE VII.

No. in Series	Date of Examination	Source of the sample	Presence or absence of *B. coli* Grammes						Do. for Streptococci Grammes					Estimated No. per grm.	
			0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	0·00001	*B. coli*	Strepto-cocci
6	Feb. 2, '04	Main channel just before creek B. Left bank	+	+	–	–	–	–	+	–	–	–	–	100	10
6	March 3, '04	Same sample but kept in cupboard in laboratory (cold)	–	–	–	–	–	–	–	–	–	–	–	less than 10	less than 10
13	Feb. 26, '04	Main channel between creeks G and P	+	+	–	–	–	–	+	–	–	–	–	100	10
13	March 4, '04	Same sample but kept in laboratory (cold) for a week	+	–	–	–	–	–	–	–	–	–	–	10	less than 10
10	Feb. 24, '04	River above the town C	+	+	–	–	–	–	+	+	+	–	–	100	1000
10	March 9	Same sample but kept in outside shed for 2 weeks	+	+	–	–	–		+	–	–	–	–	100	10
10	March 26	Same sample but kept in outside shed for 30 days	* –	–	–	–	–		+	–	–	–	–	?	10
15	March 15	100 yards above sewage outfall	+	+	+	+	–	–	+	+	+	+	–	10,000	10,000
15	March 29	Same sample but kept in outside shed for 2 weeks	+	+	–	–	–	–	+	+	–	–		100	100
15	April 5	Same sample but kept in outside shed for 3 weeks	+	+	–	–	–	–	+	+	–	–		100	100

* Organisms not typical *B. coli* isolated.

TABLE IX. *Tank I. Samples.*

Date of Examination	Presence or absence of *B. coli* in Grammes							Do. for Streptococci in Grammes				*B. coli* isolated
	1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	
Aug. 31, '04		+	+	+	+	+	−	+	+	+	−	No. 39†
Sept. 6		+	+	+	−	−	−	+	+	−	−	,, 40
,, 13		+	+	+	−	−	−	−	−	−	−	,, 41
,, 21		+	+	−	−			− *	−	−	−	,, 42
,, 27		+	+	−	−			− *	−			,, 43
Oct. 4		+	+	−	−			− *	−			,, 44
,, 11		+	−	−	−			− *	−			,, 45
,, 18		−	−	−	−			− *				—
,, 25		−	−					−				—
Nov. 1	+	−						*				No. 46
,, 8	+	−						*				,, 47
,, 15	−	−										—
,, 22	+											No. 48
,, 29	+											,, 49

* Streptococci also absent in 1 grm. of the mud.

† These numbers refer to the reference number Table VIII.

TABLE X. *Tank II. Samples.*

Date of Examination	Presence or absence of *B. coli* in Grammes							Do. of Streptococci in Grammes				*B. coli* isolated
	1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	1·0	0·1	0·01	0·001	0·0001	
Sept. 12		+	+	+	+	+	−		+	+	+	−	No. 50
,, 21		+	+	+	+	−	−		+	+	+	−	,, 51*
,, 27		+	+	+	−	−	−		+	+	−	−	,, 52
Oct. 4		+	+	+	+	−	−		+	−	−	−	,, 53
,, 11		+	+	+	−	−	−	− (0·5 g.)	−	−	−	−	,, 54
,, 18		+	+	+	−	−	−	−	−	−			,, 55
,, 25		+	+	−	−			−	−	−			,, 56
Nov. 1		+	−	−	−			−					,, 57
,, 8		+	−	−	−			−					,, 58
,, 15		+	−	−									,, 59
,, 22		−	−										—
,, 29	+	−	−										,, 60
Dec. 6	−	−											—
,, 13	−												
,, 15	−												

* On working out not a true *B. coli*.

TABLE XI. *Tank III. Samples.*

Date of Examination	Presence or absence of *B. coli* in Grammes							Do. for Streptococci in Grammes				*B. coli* isolated
	1·0	0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	
Oct. 21		+	+	+	+	+	−	+	+	+	−	No. 62
Nov. 1		+	+	+	+	−	−	+	+	−	−	,, 63
,, 15		+	+	+	+	−	−	+	−	−		,, 64
,, 22		+	+	−	−	−	−	+	+			,, 65
,, 29		+	+	−	−			+	−			,, 66
Dec. 6		−	−					+ (& in 1 g.)	−			—
,, 13*		+	−					− (absent in 1 grm.)	−			,, 67

* Mud again examined Dec. 15th for Streptococci. *Nil* found in 2 grms.

TABLE XII. *B. Typhosus in sterile mud.*

Experiment (1).

Date of Examination	Interval since experiment started	1·0 A	0·1 A	1·0 B	0·1 B	1·0 C	0·1 C
		c.c. liquid mud					
		0·1	0·01	0·001	0·0001	0·00001	0·000001
Sept. 2	—	+	+	+	+	−	−
,, 9	7 days	+	+	+	+	+	−
,, 27	25 ,,	+	+	+	+	−	−
Oct. 7	35 ,,	+	+	+	+	−	−
,, 22	50 ,,	+	+	+	−	−	−

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TABLE XII *a*. *B. Typhosus in sterile mud.*

Experiment (2).

Date of Examination	Interval since experiment started	1·0 A	0·1 A	1·0 B	0·1 B	1·0 C	0·1 C
Sept. 2	—	+	+	–	–	–	–
,, 17	15 days	+	+	+	+	*	
,, 30	28 ,,	+	+	+	+	*	
Oct. 14	42 ,,	+	+	+	–	–	–
,, 28	56 ,,	+	+	–	–	–	–

* Not examined in these dilutions.

TABLE XIII. *Group II., Experiment* (3).

Date of Examination	Presence or absence of *B. coli* Grammes						Do. for Streptococci Grammes				*B. coli* isolated
	0·1	0·01	0·001	0·0001	0·00001	0·000001	0·1	0·01	0·001	0·0001	
Oct. 29	+	+	+	+	+	–	+	+	+	–	No. 68
Nov. 5	+	+	+	+	–	–	+	+	–	–	,, 69
,, 11	+	+	–	–	–	–	+	–	–	–	,, 70
,, 18	+	+	+	–	–	–	+	+			,, 71
,, 25	+	+	–	–	–	–	–	–			,, 72
Dec. 2	+	–	–	–			–	–			,, 73
,, 9	+	+	–	–			– *				,, 74

* Streptococci also absent in 0·5 grm. of the mud.

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TABLE XIV. *Group III., Experiment* (1).

Date of Examination	Interval since experiment started	Presence or absence of *B. typhosus*						Do. for *B. coli*				
		2 loopfuls of mud	$\frac{1}{30}$ c.c. A	$\frac{1}{10}$ c.c. B	$\frac{1}{30}$ c.c. B	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1·0 c.c. B	$\frac{1}{10}$ c.c. B	1·0 c.c. C	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C
Oct. 27	—			+	+	+		+	+	−	−	
Nov. 1	5 days			+	+	+	−	+	+	+	-	−
,, 15	19 ,,		+		+		+	+	+	+	−	−
,, 22	26 ,,		+		+		+	+	+	+	+	−
Dec. 5	39 ,,		−		−		-	+	+	+	+	+ (8 colonies)
,, 9	43 ,,	−										

TABLE XV. *Group III., Experiment* (2).

Date of Examination	Interval since *B. coli* added	Presence or absence of *B. typhosus*						Do. for *B. coli*						
		2 loopfuls of mud	$\frac{1}{30}$ c.c. A	$\frac{1}{10}$ c.c. B	$\frac{1}{30}$ c.c. B	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1·0 c.c. B	$\frac{1}{10}$ c.c. B	1 c.c. C	$\frac{1}{10}$ c.c. C	$\frac{1}{30}$ c.c. C	1 c.c. D	$\frac{1}{10}$ c.c. D
Oct. 31	−			+	+	+								
Nov. 3	1 day							+	+	+	−			
,, 11	9 days				+	−	−	+	+	+	+	−		
,, 17	15 ,,		+	+	+		−	+	+	+	+	+	+	-
,, 24	22 ,,		+		+		−	+	+	+	+	+	+	-
Dec. 6	34 ,,		−		−		−	+	+	+	+	+	−	−
,, 12	40 ,,	−												