EXPERIMENTS ON THE SURVIVAL OF *B. TYPHOSUS* IN STERILISED AND UNSTERILISED SOIL.

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It was found in a series of bacteriological examinations of soil from the grounds of Queen's College, Belfast, that the ordinary saprophytic organisms present do not grow on the Drigalski-Conradi medium. Samples of soil weighing about one gramme were added to flasks containing a fluid medium of the same composition, but without the agar. When incubated at 37° C. some growth of putrefactive bacteria took place, but on plating a few loopfuls from the flask on the corresponding agar medium and again incubating for 24 hours colonies were very rarely found. No Coli-like colonies ever appeared, and the few typhoid-like colonies which were occasionally found proved on further examination to be cocci, frequently *Micrococcus tetragenus*. On the other hand, if a loopful of *B. coli* or *B. typhosus* were added to the flask along with the soil the plates made after incubation showed numerous colonies of these bacilli.

In view of these results it appeared to be worth while to investigate the survival of the typhoid bacillus in soil with the aid of the Drigalski medium.

The soil used was taken at a depth of 3 to 4 inches from under grass in the College grounds. In the first set of observations the soil was placed in wide necked glass stoppered bottles; of these some were sterilised by steam in the autoclave for half-an-hour at 120° C., others were left unsterilised.

Pure cultures of *B. typhosus* and *B. coli* were isolated from the fæces of a typhoid patient on Nov. 17th, 1904, and these were used to inoculate

the soils in the following manner. Surface agar cultures made in Petri dishes and incubated at 37° C. for 24 hours were emulsified in a few cubic centimetres of sterile water and sprinkled as evenly as possible over the surface of the soil, one plate culture being used for each sample. Thus no nutrient material was added to the soil along with the bacilli, a point of importance which has been overlooked in some previous investigations. The bottles were covered with brown paper to exclude the light; some were kept at a constant temperature of 20° C., others were exposed to the natural outside temperatures. Enumerations of the bacilli were made from time to time in the following manner. A small quantity—approximately 1 gramme—of the soil was removed from the surface of the sample with a sterile platinum spud and transferred to a flask containing 100 c.c. sterile water. The flask was weighed before and after the addition of the soil and the amount taken thus ascertained.

The flask was thoroughly shaken and further dilutions of 1 in 100, 1 in 1000, etc. made by transferring 1 c.c. from the flask to a tube containing 9 c.c. sterile water, 1 c.c. of this to another similar tube and so on.

By means of a capillary pipette, delivering 30 drops to 1 c.c., 1/30 c.c. or 1/15 c.c. of the necessary dilutions was added to the surface of Drigalski plates and spread by means of a sterile glass rod bent at right angles. The plates were counted after 24 hours incubation and from the number of colonies appearing a sufficiently approximate estimate of the number of bacilli in 1 gramme of soil could be made. As the number of bacilli decreased the method of isolation indicated in the first paragraph was used. When typhoid-like colonies appeared these were subcultured and tested on all the usual media and by agglutination.

Only a summary of the final results of the first set of experiments is given here. The enumerations do not differ materially from those of the later experiments which are given in full.

Experiments started November 28th, 1904.

Unsterilised soil inoculated with emulsion of *B. typhosus*, moisture 35 %.

	Temperature	*B. typhosus* present	Absent
Exp. (1)	Outside	After 50 days	In 61 days
,, (2)	20° C.	,, 42 ,,	,, 50 ,,

Sterilised soil inoculated with emulsion of *B. typhosus*, moisture 34 %.

	Temperature	*B. typhosus* present	Absent
Exp. (3)	Outside	After ? days	In 11 days
,, (4)	20° C.	,, ? ,,	,, 11 ,,

Unsterilised soil inoculated with emulsions of *B. coli* and *B. typhosus*, moisture 35 %.

Exp. (5) Outside. *B. coli* present in large numbers after 42 days.
B. typhosus not isolated.

Exp. (6) 20° C. *B. coli* present in large numbers after 50 days.
B. typhosus not isolated.

Sterilised soil inoculated with emulsions of *B. coli* and *B. typhosus*, moisture 34 %.

Exp. (7) Outside. *B. coli* present after 13 days, absent in 21 days.
B. typhosus not isolated.

Exp. (8) At 20° C. *B. coli* present after 50 days.
B. typhosus present after 9 days, not found later.

In all the experiments the typhoid bacilli decreased in numbers fairly rapidly, but as will be seen from the above tables, they survived five times as long in the natural soil as in that which had been subjected to sterilisation. This result was contrary to all expectation. In the soils inoculated with a mixture of *B. coli* and *B. typhosus* no conclusions can be drawn with regard to the latter. The results for *B. coli* are not uniform. In the natural soils the bacillus was present in large numbers at the end of 50 days and was showing no signs of dying out. In one of the sterilised samples it had died out in 21 days. In the other, which was kept at a higher temperature, it at first showed a rapid decrease in numbers but later apparently it managed to establish itself. We may conclude that for the *B. coli* also this soil, when sterilised, forms a less favourable medium than it does in its natural condition.

To confirm these results a new set of experiments was started on March 11th, 1905. The moisture present in the soils of the first series (34—35 %) is higher than the average soil moisture during the winter months, which was 27·4 % in 15 samples taken between October and January. The excessive moisture, together with the fact that the samples were kept in stoppered bottles, led to the growth of moulds in some of the samples.

In the next experiments therefore the conditions were altered so as to secure a lower percentage of moisture and better aëration. The soil used was taken from the same place as before.

Different portions of this were treated as follows:—(1) Part was dried at a temperature of from 30° to 40° C. in a current of air. (2) Part was sterilised in the autoclave at 115° C. for 20 minutes and then dried in the hot air oven at 100° C. (3) Part was dried in the hot air oven at 90° to 100° C. without previous sterilisation. Each portion was then powdered in a mortar and 120 grammes placed in the bottom of a wide glass jar with a loosely fitting cover. The depth of earth in

each jar was about 1 inch. The emulsions of typhoid bacilli, made from agar plates as before, were added in the amount of 40 c.c. to each jar and a moisture of approximately 25 % was thus obtained.

The emulsions were made with sterile tap water except in one case where sewage sterilised by filtration through porcelain was used instead. Observations were made with *B. typhosus* only and the same strain was used as in the previous experiments. The jars were covered with brown paper to exclude light and all were kept at outside temperatures.

The enumerations and the isolation of the typhoid bacillus were carried out as before. The samples for examination were taken from the surface except where otherwise stated.

Experiment No. 9. Unsterilised soil dried at 30°—40° C. inoculated with emulsion of *B. typhosus*, March 11th, 1905.

Date	Days	*B. typhosus* per gramme	Moisture
Mar. 11		84,000,000 (at least)	25 %
,, 14	3	132,000,000	
,, 17	6	156,000,000	
,, 20	9	52,200,000 (at least)	
,, 24	13	1,200,000	
,, 28	17	255,000 (deep sample)	
,, 31	20	9,000,000 (at least)	25 %
Apr. 3	23	540,000 (deep sample)	
,, 5	25	less than 3,000 (none found)	
,, 10	30	,, ,, 600 ,, ,,	
,, 11	31	present in ½ grm.	
,, 26	46	present in 1 ,,	
May 13	63	absent in 1 ,,	
,, 21	71	present in 1 ,,	
June 1	82	absent in 1 grm. (Also on June 5 and 12.)	

Experiment No. 10. Sterilised soil dried at 100° C. inoculated with emulsion of *B. typhosus*, March 11th, 1905.

Date	Days	*B. typhosus* per gramme	Moisture
Mar. 11		152,000,000	26 %
,, 14	3	28,500,000	
,, 17	6	less than 300,000 (none found)	
,, 18	7	1,500	
,, 20	9	100	
,, 22	11	absent in 1 grm.	
,, 24	13	absent in 1 ,, (Also on May 13th.)	

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Experiment No. 11. Unsterilised soil dried at 30°—40° C. inoculated with emulsion of *B. typhosus* in sterile sewage, March 11th, 1905.

Date	Days	*B. typhosus* per gramme	Moisture
Mar. 11		124,000,000	23 %
,, 14	3	144,000,000	
,, 17	6	180,000,000	
,, 20	9	102,600,000	
,, 24	13	2,400,000 (deep sample)	
,, 28	17	4,500,000 ,, ,,	
,, 31	20	at least 18,000,000	20 %
Apr. 3	23	300,000 (deep sample)	
,, 5	25	219,000	
,, 10	30	1,800	
,, 26	46	present in 1 grm.	
May 13	63	present in 1 ,,	
,, 21	71	present in 1 ,,	
June 1	82	absent in 1 ,,	
,, 5	86	present in 1 ,,	
,, 12	93	absent in 1 grm. (Also on June 19th.)	

Experiment No. 12. Soil dried at 90°—100° C., bacteria present after drying at least 24,000 per gramme, inoculated with emulsion of *B. typhosus*, March 23rd, 1905.

Date	Days	*B. typhosus* per gramme	Moisture
Mar. 23		180,000,000	24·6 %
,, 28	5	36,000,000	
,, 31	8	at least 150,000,000	23 %
Apr. 3	11	990,000 (deep sample)	
,, 5	13	3,000,000	
,, 10	18	16,000	
,, 26	34	present in 1 grm.	
May 13	51	present in 1 ,,	
,, 21	59	present in 1 ,,	
June 1	70	absent in 1 ,,	
,, 5	74	present in 1 ,,	
,, 12	81	absent in 1 grm. (Also on June 19th.)	

In the sterilised soil (Exp. No. 10) the typhoid bacillus disappeared in 11 days just as in the previous experiments (Nos. 3 and 4), while in the unsterilised soils it survived for periods of 71, 74, and 86 days respectively. The addition of sewage in Exp. No. 11 had little effect in prolonging its survival. Even in the unsterilised soils there is a progressive decrease in the number of the bacilli, this being most rapid after about the 20th day.

The remarkable rapidity with which the typhoid bacillus dies out in this soil when it has been subjected to sterilisation by steam under pressure calls for some explanation. Exp. No. 12 shows that the effect is not produced by dry heat at 100° C. It appeared most probable that

the effect is due to the production during sterilisation of substances which have a bactericidal action, as for example the formation of acids by hydrolysis. No change, however, in the reaction of the soil washings to phenol-phthalein could be made out, and it seemed possible that the presence of the soil bacteria might have a favourable action on the survival of the typhoid bacillus.

To test this question a new series of experiments was started on October 28th. The soil was taken from a different part of the College grounds also under grass. It differed in appearance from that used in the experiments already described, being redder in colour and more sandy. One portion was dried at 80° C. for two or three hours, another was sterilised for 20 minutes at 115° C. in the autoclave and then dried at 80° C. In each case 150 grm. of the dried soil was placed in the glass jar and 50 c.c. of typhoid emulsion distributed as evenly as possible over the surface as in the former experiments.

One sample of the sterilised soil received along with the typhoid bacilli emulsions made from cultures of bacilli isolated from the soil.

Two cultures (*A* and *B*) of bacilli belonging to the "subtilis" group were used. *A* shows a spreading filamentous growth on agar, *B* a tough white growth with little tendency to spread. Pieces of the growths were ground up in an agate mortar with a little water, after dilution the emulsion was centrifugalised to get rid of the larger masses of growth and 5 c.c. were added to the 50 c.c. of typhoid emulsion. The same culture of *B. typhosus* was used and the technique was in every respect the same as before. In addition, enumerations of the spores present were made on gelatin plates, a temperature of 80° C. for 10 minutes being used to kill off non-sporing forms. After the typhoid bacilli had died out counts of the total bacteria growing on gelatin were made.

Experiment No. 13. Soil dried at 80° C. inoculated with emulsion of *B. typhosus*, Oct. 28th, 1905.

Date	Day	*B. typhosus* per gramme	Spores per gramme	Moisture
Oct. 29	1	250,000,000	8,200	26·7 %
Nov. 3	6	8,000,000		
,, 7	10	25,000,000	6,000	25·1 %
,, 14	17	1,400,000		25 %
,, 23	26	less than 2,000	at least 100,000	
,, 28	31	12,000		
Dec. 7	40	less than 200	400,000	
Jan. 5	69	present in 1 grm.		25·1 %
,, 19	83	absent in 1 grm.	3,320,000	
		(Total on gelatin)	20,000,000	

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Experiment No. 14. Sterilised soil inoculated with emulsion of *B. typhosus*, Oct. 28th, 1905.

Date	Day	*B. typhosus* per gramme	Moisture
Oct. 29	1	80,000,000	27·1 %
Nov. 3	6	8,000,000	
,, 7	10	20,000,000	28 %
,, 14	17	10,000,000	22·4 %
,, 23	26	60,000	
Dec. 7	40	600	
Jan. 5	69	present in 1 grm.	20·4 %
,, 19	83	absent in 1 grm.	

Experiment No. 15. Sterilised soil inoculated with emulsions of *B. typhosus* and soil bacteria, Oct. 28th, 1905.

Date	Day	*B. typhosus* per gramme	Spores per gramme	Moisture
Oct. 29	1	250,000,000	less than 100	29·2 %
Nov. 3	6	1,800,000		
,, 7	10	12,000,000	12,500	28·5 %
,, 14	17	4,000,000	8,000	
,, 23	26	30,000	5,000	
Dec. 7	40	400	60,000	
Jan. 8	72	present in 1 grm.		23·9 %
,, 19	83	absent in 1 grm. (Total on gelatin)	142,000 11,200,000	

In this series of experiments the typhoid bacillus died out at exactly the same rate in all three samples, the time of survival corresponding to that of the unsterilised soils of the previous series. In this case therefore sterilisation of the soil had no inimical action on the survival of the bacillus, a marked contrast to the results of Exps. Nos. 3, 4, and 10. The addition of soil bacteria to the sterilised soil had no effect on the survival of the typhoid bacillus.

The difference in the behaviour of the sterilised soil in Exp. No. 14 can only be explained by differences in its chemical composition. It appears therefore that in some samples of soil, but not in all, substances are produced during sterilisation which have a bactericidal effect on the typhoid bacillus. The matter requires further investigation.

The progressive increase in the natural soil bacteria in Exps. Nos. 13 and 15, corresponding with the decrease in the typhoid bacilli, is interesting and shows that the soil bacteria are able to utilise the dead typhoid bacilli as food supply. The numbers reached at the close of the experiments are far in excess of those ordinarily present in uncontaminated soil and indicate the presence of organic material suitable for the growth of saprophytes. Active growth of the soil organisms is also shown by the high proportion of vegetative forms in the last counts.

That the soil organisms have not, as has been supposed, an antagonistic action towards the typhoid bacillus is shown by the fact that the latter survived as long in Exps. Nos. 13 and 15 as in No. 14.

Sidney Martin (1897—1900) in an elaborate investigation extending over a number of years, found that in sterilised virgin soils the typhoid bacillus died out rapidly, while in sterilised contaminated soils growth occurred and the bacillus could be recovered after 400 days. His inoculations were made as a rule with broth cultures. In the few experiments where the bacillus was added to the soil without nutrient broth the evidence of growth is not very conclusive. Where nutrient broth is added to the soil even in small amounts the conclusion that the soil *per se* forms a suitable medium for the growth of the bacillus is not warranted; all that can be claimed is that the soil in question has no inimical action on the bacillus. The destructive action of the sterilised virgin soil on the typhoid bacillus even when added in broth culture was probably due, as my results show, to the production of bactericidal substances during sterilisation. This soil was not examined in the natural condition. Contaminated soils, proved when sterilised and inoculated with broth cultures to be capable of supporting the life of the bacillus, were examined without previous sterilisation. When inoculated with broth cultures of *B. typhosus* and incubated at 37° C., in five experiments, the bacillus was recovered once after 50 days, once after 24 hours, and in the remainder not at all. When the samples were kept at lower temperatures the typhoid bacillus could be recovered more easily but never after 12 days. Martin concludes that the saprophytic bacteria of the soil have an antagonistic action towards the typhoid bacillus. This conclusion is not warranted, as the difficulties in the detection of the typhoid bacillus in the presence of a large number of saprophytes are almost insuperable by the methods used. The nutrient broth added to the soil allowed of rapid multiplication of organisms of the "subtilis" group. Flügge (1895) has shown that these organisms grow with enormous rapidity at temperatures above 22° C. when placed under favourable conditions, while below that temperature their growth is comparatively slow. This accounts for the greater ease with which the typhoid bacillus was recovered when the soils were kept at a low temperature (2° C.—12° C.). Martin's further experiments show that the products of putrefaction have a bactericidal action on the typhoid bacillus, but these products only occur in the presence of an abundant supply of nitrogenous material. In an ordinary soil the putrefactive bacteria are in a quiescent condition, a large proportion being present in the form of spores. Exp.

No. 11 shows that the addition of filtered sewage does not supply enough organic material to enable the putrefactive bacteria to exert a harmful influence on the typhoid bacillus. In this case the typhoid bacillus was recovered after 86 days, that is, it survived rather longer than in the control experiment with uncontaminated soil. The progressive increase in the number of saprophytes in Exps. Nos. 13 and 15 is not to be interpreted as evidence of an antagonistic action on their part towards the typhoid bacillus, as the latter died off at exactly the same rate in the sterilised sample. In this connection it may be noted that Pfuhl (1899) found that the typhoid bacillus is capable of growing on potato and spreading into its substance even in the presence of *B. subtilis*.

Whether or not the typhoid bacillus can to any extent multiply in a natural soil has not been definitely determined. There is no evidence of such increase in my experiments, but the inoculations were made with such large numbers of bacilli that a slight growth might not have been noted. The constant decrease in numbers under the conditions of the experiments makes it appear very unlikely that any growth does take place.

The results of others who have investigated the survival of *B. typhosus* in soil may be summarised here. Grancher and Deschamps (1889) inoculated soil in a natural condition with broth cultures of *B. typhosus* and recovered the bacillus after five and a half months.

Almquist (1893) recovered the bacillus from a sterile mixture of sand and dung after "a considerable time," while in pure sand it died out rapidly. No exact data are given; broth cultures were used. Dempster (1894) using small quantities of sterilised soil and emulsions of *B. typhosus* in water recovered the bacillus up to the 18th day. In sterilised peat the bacillus disappeared in 24 hours. He considers that in soils which have not a definite destructive action on the bacillus the time of survival is chiefly dependent on the amount of moisture present.

Robertson (1898) added broth cultures of *B. typhosus* to patches of ground from which the grass had been removed. In one set of observations he recovered the bacillus after four months, in another where frequent additions of nutrient material were made to the soil the bacillus survived for ten months.

Rullman (1901) following Martin's methods showed that the typhoid bacillus can spread in some soils to which it has been added in the form of broth cultures. He recovered the bacillus after 216 days in sterilised, and after 100 days in unsterilised samples.

Lorrain Smith (1903) investigated the survival of *B. typhosus* in soils

from various sources both after sterilisation and in the natural condition. He used emulsions made from growths on potato, care being taken not to add any nutrient material along with the bacilli. He also enumerated the typhoid bacilli present in the samples from time to time using phenolated gelatin plates. The counts showed a rapid diminution in the numbers of typhoid bacilli. The longest period of survival observed in the unsterilised soils was 21 days and the average was 15 days. In the sterilised soils inoculated as mentioned above, the average survival was 16 days and the maximum 25 days. For purposes of comparison samples of sterilised soil to which amounts of nutrient broth varying from 10 % to 0·01 % had been added were inoculated with *B. typhosus*. Here the bacilli died out at the same rate in all the samples and were not recovered after 23 days. The samples in this case were taken from the grounds of Queen's College and were therefore of the same nature as those used by the present writer. The results show a directly bactericidal action of the *sterilised* soil. Unfortunately control experiments with unsterilised soil were not in this case made. Another experiment of Lorrain Smith's is given here in detail for comparison with those of the present paper.

"Experiment 22. Soil from the College grounds dried, sifted, and saturated with sewage; again dried and steam sterilised. It was then inoculated with a culture of typhoid bacilli.

Date	Day	Moisture	No. of bacilli per gramme
29/1/03	1	21·5 %	1,075,000
30	2		105,000
1/2/03	4		27,000
3	6		38,000
5	8		4,300
7	10		8,000
10	13		8,700
12	15		1,200
16	17		840
21	24		550
23	26		435
23/3/03	55		Less than 100"

The time of survival in this sterilised soil corresponds with that found in Exp. No. 14. In this case previous sterilisation did not render the soil specially unsuitable for the survival of the typhoid bacillus as in Exps. Nos. 3, 4, and 10.

I wish here to express my thanks to Prof. Lorrain Smith for much kind help and advice.

SUMMARY OF CONCLUSIONS.

1. The typhoid bacillus can survive in natural soil in large numbers for about 20 days and is still present in a living condition after 70 to 80 days.

2. There is no evidence that the typhoid bacillus is capable of multiplying and leading a saprophytic existence in ordinary soil.

3. In some samples of soil, but not in all, the typhoid bacillus dies out much more rapidly (in 11 days) if the soil has previously been subjected to sterilisation by steam under pressure. This is apparently due to the production of bactericidal substances during sterilisation.

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