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THE VITALITY AND VIABILITY OF STREPTOCOCCI IN WATER.

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(*From the Somerset County Public Health Laboratory.*)

(With 4 Text-figures.)

IN an earlier paper from this Laboratory the significance of streptococci in water supplies was dealt with from the point of view of the comparative occurrence of streptococci and *B. coli* in different classes of waters. It was demonstrated that these two groups of organisms corresponded fairly closely as regards their numerical presence in water supplies. It was pointed out that "while we do not know enough about the varying vitality and distribution of streptococci to say whether the presence of certain strains may or may not be disregarded as evidence of excretal contamination it is, in general, reliable to assume that streptococci in large numbers are only present in waters from unsatisfactory sources."

The bacteriologist who has to report upon water supplies is always seeking for data, which as yet he does not possess, which shall give him information upon two points—whether the excretal pollution detected is animal or human and as to the date and recency of any contamination. The series of experiments recorded here were designed to throw light upon the second point and more particularly from the point of view of streptococci in water.

While Houston, Horrocks and others have carried out investigations upon the viability of isolated streptococci in water or sewage and Prescott and Baker have studied the relative growth of *B. coli* and sewage streptococci from polluted waters in glucose broth we are unaware of any work dealing with the comparative vitality of *B. coli* and streptococci in water under nearly natural conditions.

It seemed to us that experiments upon these lines would supply data of value in determining the relative significance of these organisms in water.

Methods of investigation.

To study the comparative viability of streptococci and *B. coli* in water cylindrical earthenware tanks were used each of a diameter of 12 inches and a capacity of about 50 litres. They were obtained by using large drainpipes with a cement bottom but open at the top. These tanks were kept in the open air, lightly covered to prevent rain or dust access but with free air admission. The temperature of the water was recorded daily. About 40 litres of unsterilised tap-water (a bacteriologically good water) was put into each tank to which was added varying quantities of sewage or excreta. Preliminary experiments with samples of sewage and excreta showed great variations in their *B. coli* and streptococcus content so that it was not possible to add always the same amount. This however was quite immaterial, the aim being to have about 1000 *B. coli* or streptococci per c.c. of the final mixture without making it a nutritive material. In view of the comparatively fewer streptococci in animal excreta it was found better in the later experiments to first mix a definite amount of the excreta with a little sterile water, allow the coarser particles to separate and then add the bacterially rich upper emulsion to the tank water. In this way the majority of the bacteria were added with comparatively little organic matter.

After the addition of the excrementitious material the mixture was very thoroughly stirred and the first sample collected. In the first three recorded experiments (I, III, IV) the water was stirred before each sample was taken. In the other experiments no further stirring was resorted to until the end of 4 weeks. In a number of cases duplicate samples were collected one just before, the other just after, stirring, to study how far any diminution was due to sedimentation without loss of viability.

Contrary to our expectations very little difference was made by stirring the water at the end of 4 weeks. In Exps. IX, X, XI, or XV, practically no increase in either organism was observed. In Exp. XVI some slight increase in the number of streptococci was recorded, while in Exp. XIII there was a considerable increase in the number of *B. coli*, but in this experiment there was an appreciable amount of deposit. Our results show that, in the absence of sediment, these bacteria do not appreciably precipitate and remain alive, even in perfectly still water.

In a few of the experiments when one type persisted long after the other had died out, at the end of 4 weeks or longer (but never earlier) the completion of the experiment was studied by transferring part of the water to a large sterile bottle kept under similar conditions.

To examine the tank water samples were collected in sterile bottles and examined immediately.

The *B. coli* enumerations were made by the usual lactose bile salt broth method using lactose bile salt neutral red agar for plating out. The streptococcus enumerations were made by the method we have described elsewhere, i.e. by adding definite fractions of the water to neutral red broth (single or double strength) and examining for streptococci in hanging drop preparations after incubation for 40–48 hours at 37° C. Only cocci in quite definite chains were taken as evidence of the presence of streptococci while negative results were only recorded after repeated examinations. In doubtful cases the deposit was centrifugalised and stained.

To avoid wide spacing of results amounts intermediate to the usual decimal dilutions were also employed, *i.e.* 0·03, 0·3, etc. as well as 0·01, 0·1, 1·0. Such intermediate amounts approximate closely to the mean in geometrical progression between 10 and 1 c.c., 1 and 0·1 c.c., etc. (viz. 3·16, 0·316, etc.), and the quantities taken for examination are thus uniformly spaced, each quantity being approximately one-third the quantity preceding it in the series.

The experiments upon the viability of individual strains of streptococci and *B. coli* were carried out in stoppered bottles containing one litre of tap-water, kept in diffuse daylight at room temperatures, a cool North room being utilised. The tap-water used was a very hard one so, in order to avoid mineral sediment, the water was first boiled and the precipitated calcium carbonate filtered off before the sterilisation.

We desire in particular to point out that the amounts of excrementitious material added were in no case sufficient to convert the water into a nutrient material. This is a matter of considerable importance as we wished our experiments to be under strictly practical conditions. The amounts added were not more than might readily occur under natural conditions of rather gross pollution. Free and albuminoid ammonia determinations were carried out in a number of cases at the start of the experiment and they showed that the organic matter added was not considerable. For example:

Exp. XV.	Cow excreta and water	Free = 0·004	Albuminoid = 0·032
Exp. XVI.	Sewage excreta and water	,, = 0·008	,, = 0·007
Exp. XXII.	Human excreta and water	,, = 0·001	,, = 0·008
Exp. XXIII.	Cow ,, ,,	,, = 0·026	,, = 0·085

Exps. II, XII and XIV did not give satisfactory relative numbers of the two organisms so were not followed out and are not recorded.

Details of the experiments.

Group A. Comparative viability in water of B. coli and streptococci derived from human excreta.

Four experiments were carried out. The actual figures of the numbers present per c.c. are set out in Table I and the percentage survival in Table II.

TABLE I. (*Numbers per c.c. of the water.*)

Examination intervals	Exp. IX		Exp. X		Exp. XI		Exp. XXI	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	1000–3000	30–100	100–300	100–300	1000–3000	100–300	30–100	1000–3000
3 days	100–300	30–100	300–1000	300–1000	1000–3000	30–100	10–30	30–100
7 ,,	10–30	3–10	10–30	1–3	300–1000	10–30	3–10	100–300
2 weeks	10–30	absent*	0·03	absent	3–10	0·1	1–3	1–3
3 ,,	1–3	,,	0·03	,,	3–10	0·03	0·03	1–3
4 ,,	0·3	,,	0·03	,,	3–10	absent	absent	0·03
4 ,, (after stirring)	1–3	,,	0·03 (3 weeks)	0·03 (3 weeks)	3–10	0·03	—	—
5 weeks	0·1	—	absent	absent	0·3	0·03	absent	absent
6 ,,	0·03	—	—	—	0·1	absent	—	—
7 ,,	absent*	—	—	—	absent	,,	—	—

* *absent* = absent from 30 c.c.

Exp. IX. 0·5 grm. normal human excreta to 40 litres tap-water. Sept.–Nov. average temperature 52° F.

Exp. X. Excreta from convalescent case of paratyphoid fever. Emulsion of 4 grm. in 100 c.c. sterile water. After a few minutes settlement two-thirds of supernatant fluid added to 40 litres tap-water. Nov. and Dec. average temperature 40·1° F.

Exp. XI. Two grm. excreta from another convalescent paratyphoid fever case to about 40 litres tap-water. Nov., Dec., Jan. average temperature 37° F.

Exp. XXI. 3·0 grm. excreta from a suspected typhoid bacillus carrier (no typhoid bacilli found) added to 40 litres tap-water. March and April average temperature 42° F.

TABLE II. *Percentage survival.*

Examination intervals	Exp. IX		Exp. X		Exp. XI		Exp. XXI	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	100	100	100	100	100	100	100	100
3 days	10	100	300	300	100	30	33	3
7 ,,	1	10	10	1	30	10	10	10
2 weeks	1	0	0·03	0	0·3	0·1	3	0·1
3 ,,	0·1	0	0·03	0	0·3	0·03	0·1	0·1
4 ,,	0·03	0	0·03	0	0·3	0	0	0·003
4 ,, (after stirring)	0·1	0	0·03	0·03	0·3	0·03	—	—
5 weeks	0·01	—	0	0	0·03	0·03	0	0
6 ,,	0·003	—	—	—	0·01	0	—	—
7 ,,	0	—	—	—	0	0	—	—

Remarks. All four experiments show a rapid diminution in the number of both groups of organisms which is so marked that at the end of as short a period as two weeks they are either absent or present in

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insignificant numbers only (except in one experiment). This elimination does not markedly favour one group more than the other, the chief difference being that the final disappearance of *B. coli*, as shown by its absence from 30 c.c., is usually much more protracted than for streptococci.

Group B. Comparative viability in water of B. coli and streptococci derived from animal excreta.

Three experiments, the results being shown in Tables III and IV.

TABLE III.

Examination intervals	Exp. IV		Exp. XIII		Exp. XV	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	1000–3000	1–3	100–300	100–300	30–100	100–1000
3 days	—	—	30–100	0·3	1–3	3–10
7 „	300–1000	0·03	30–100	0·3	3–10	10–30
2 weeks	100–300	absent	10–30	0·1	1–3	30–100
3 „	30–100	„	absent	absent	0·1	0·03
4 „	3–10	„	„	„	0·03	0·03
4 „ (after stirring)	—	—	1–3	„	—	—
5 weeks	1–3	absent	0·3	„	0·03	absent
6 „	0·3*	—	0·03	—	0·03	„
7 „	—	—	absent	—	absent	—

* This organism survived for long periods. It was found present in 10 c.c. of the water at the end of 11 and 16 weeks respectively. In its cultural characters it fermented lactose only slightly but in other respects was typical producing indol, clotting milk, etc. It showed capsule formation.

Exp. IV. 2·0 grm. sheep excreta to 41 litres tap-water. May to Sept. 1916. Average temperature 61° F.

Exp. XIII. 30 grm. of cow excreta emulsified with water and strained through muslin. The filtrate added to about 40 litres tap-water. Dec. 1916 and Jan. 1917. Average temperature 48° F.

Exp. XV. 30 grm. of cow excreta emulsified with water and the filtrate after straining through muslin added to about 40 litres tap-water. Jan. and Feb. 1917. Average temperature 33° F.

TABLE IV. *Percentage survival.*

Examination intervals	Exp. IV		Exp. XIII		Exp. XV	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	100	100	100	100	100	100
3 days	—	—	30	0·3	3	3
7 „	30	3	30	0·3	10	10
2 weeks	10	0	10	0·1	3	30
3 „	3	0	0	0	0·3	0·03
4 „	0·3	0	0	0	0·1	0·03
4 „ (after stirring)	—	—	1	0	—	—
5 „	0·1	—	0·3	—	0·1	0
6 „	0·03	—	0·03	—	0·1	0
7 „	—	—	0	—	0	—

Remarks. As for Group A there is a rapid diminution of both *B. coli* and streptococci but with a more prolonged mere persistence of *B. coli* in small numbers. The survival was rather longer than with human excreta, the results after 3 weeks being more nearly comparable to those with human excreta after 2 weeks.

It is interesting to note that the surviving *B. coli* in both Exp. XIII and XV was of the type described by Clark and Lubbs, having its origin in grain, and distinguishable from the normal excretal type of *B. coli* by the low final concentration of hydrogen ions produced in glucose hydrogen di-potassium phosphate peptone water medium. They are called by these authors "high ratio" organisms since the ratio CO_2 : H produced in glucose media exceeds that of normal *B. coli*.

Group C. Comparative viability in water of B. coli and streptococci derived from sewage.

Four experiments, the results being shown in Tables V and VI.

TABLE V.

Examination intervals	Exp. I		Exp. III		Exp. XVI		Exp. XX	
	B. coli	Streptococci	*B. coli*	Streptococci	*B. coli*	Streptococci	*B. coli*	Streptococci
Start	100–300	10–30	30–100	30–100	100–300	100–300	300–1000	300–1000
3 days	30–100	3–10	100–300	30–100	over 1000	300–1000	100–300	300–1000
7 „	0·3	0·03	3–10	0·3	100–300	300–1000	10–30	100–300
2 weeks	0·1	0·03	0·1	0·1	30–100	3–10	3–10	1–3
3 „	0·03	0·03	0·1	0·1	10–30	3–10	0·3	0·03
4 „	0·03	0·03	0·03	0	1–3	3–10	0·3	0·03
4 „ (after stirring)	—	—	*0*	*0*	—	—	—	—
5 weeks	0	0·03	0	0	0·3	10–30	1–3	0·03
6 „	0	0	—	—	0·1	3–10	0·03	0
7 „	0	0	—	—	0·03	0·03	0	0
8 „	—	—	—	—	0	0·1	—	—
9 „	—	—	—	—	—	—	—	—

Exp. I. 165 c.c. domestic raw sewage added to 41 litres tap-water. Feb., March, April, 1916. Average temperature 38° F.

Exp. III. 100 c.c. domestic raw sewage to 41 litres tap-water. April and May, 1916. Average temperature 53° F.

Exp. XVI. 200 c.c. of a mixture of several sewage samples added to 40 litres of tap-water. Jan., Feb., March, 1917. During part of this period the tank water was frozen hard and only a little water could be obtained for sampling. The tank was cracked by the frost but several litres of the water were obtained and transferred to a sterile bottle and the experiment continued in a cold room. Average temperature first three weeks =32·5° F. afterwards 45° F.

Exp. XX. 200 c.c. of a mixture of two sewage samples added to 40 litres of tap-water. Feb., March, April, 1917. Average temperature 41° F.

At the end of 4 weeks and 5 weeks the *B. coli* were plated out and on each occasion both excretal *B. coli* and also the high ratio (CO_2: H) *B. coli* of Clark and Lubbs were isolated.

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TABLE VI. *Percentage survival.*

Examination intervals	Exp. I		Exp. III		Exp. XVI		Exp. XX	
	B. coli	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci	*B. coli*	Strepto-cocci
Start	100	100	100	100	100	100	100	100
3 days	30	30	300	100	1000	300	33	100
7 ,,	0·3	0·3	10	1	100	300	3	33
2 weeks	0·1	0·3	0·3	0·3	30	3	1	0·3
3 ,,	0·03	0·3	0·3	0·3	10	3	0·1	0·01
4 ,,	0·03	0·3	0·1	0	1	3	0·1	0·01
5 ,,	0	0·3	0	0	0·3	10	0·3	0·01
6 ,,	0	0	—	—	0·1	3	0·01	0
7 ,,	0	0	—	—	0·03	0·03	0	0
8 ,,	—	—	—	—	0	0·1	—	—

Remarks. While the results follow the general features of the two other groups of experiments the total elimination, particularly of the streptococci, is not quite so rapid. In three out of the four experiments the number of streptococci and *B. coli* were the same at the start, making the comparative alteration specially interesting. The decline was very similar for both organisms and the parallelism close.

The frozen condition caused a prolonged survival of both *B. coli* and streptococci in Exp. XVI.

Group D. Experiments carried out with isolated strains.

Exp. V. A mixture of 10 streptococci, 6 isolated from bovine, 4 from human excreta, and 18 *B. coli* strains derived 10 from milk, 5 from sheep, 2 ox, 1 from human excreta kept in sterile tap-water. The strains were grown in broth and 4 loopfuls of each culture were added to 2 litres of tap-water in a stoppered bottle. The 112 loopfuls (about 0·2 c.c. of broth) added very little organic matter. The bottle was kept in the dark at room temperature.

The results obtained were as follows:

Examination intervals	*B. coli* (per c.c.)	Streptococci (per c.c.)
Start	1000–10,000	100–1000
1 week	10,000–100,000	100–1000
2 weeks	over 100,000	100–1000
3 ,,	100,000–1,000,000	10–100
4 ,,	over 1,000,000	0·1–1
5 ,,	,, 10,000,000	absent (from 10 c.c.)
6 ,,	1,000,000–10,000,000	,, ,,
7 ,,	10,000–100,000	,, ,,
8 ,,	100,000–1,000,000	,, ,,
9 ,,	10,000–100,000	,, ,,

Experiment not continued further. It ran from June 8th to August 18th, 1916 so that the temperature was considerable over part of this period. It was frequently between 60 and 70° F.

Exp. VI. One of the *B. coli* strains from Exp. V was isolated a month after the start of that experiment and its viability in water separately tested. 0·1 c.c. of a 24 hours' old peptone water culture was added to half a litre of sterile tap-water in flask. Kept in the laboratory in diffused light. Average temperature 60–70° F.

At start	42,000 per c.c.
End of 5 days	3,500,000 ,,
,, 12 ,,	6,000,000 ,,
,, 36 ,,	4,800,000 ,,

Although amount of nutrient material present was quite negligible the organism showed marked powers of multiplication. In its cultural characters it agreed with the ordinary excretal *B. coli* strains fermenting glucose and lactose, producing indol, clotting milk in 2 days and growing as a bluish translucent growth without liquefaction, upon sloped nutrient gelatine.

Exp. VII. Another *B. coli* strain isolated from human excreta kept in sterile tap-water; 0·1 c.c. of a 20 hours' peptone water culture being added to 1 litre of sterile tap-water and kept at room temperature in a stoppered bottle.

	At start 12,700 per c.c. (July 27th).		
After 6 days	102,000 per c.c.	After 21 days	100,000 per c.c.
,, 13 ,,	112,000 ,,	,, 36 ,,	162,000 ,,

The organism was still present in 1 c.c. after 147 days but was absent in 10 c.c. 40 days later.

Exp. VIII. Six streptococcus strains all recently isolated from human excreta kept in sterile water in stoppered bottle at room temperature. All short chain forms. 0·1 c.c. of a 24 hours' culture in broth added in each case.

At start	3120 per c.c.
After 8 days	2900 ,,
,, 14 ,,	1550 ,,

At the end of 21 days the number per c.c. was 10,000 but a contaminating bacillus was present so the results were unreliable and the experiment was discontinued.

Exp. XVII. A streptococcus strain isolated from cow excreta and kept in sterile tap-water in stoppered bottle at room temperature. 0·2 c.c. of a 20 hours' broth culture added to 1 litre water.

	At start (Jan. 17th) 46,000 per c.c.		
After 3 days	20,000 per c.c.	After 9 days	50 per c.c.
,, 7 ,,	200 ,,	,, 14 ,,	absent from 1 c.c.

Exp. XVIII. A streptococcus isolated from the fresh sewage used for Exp. XVI. 0·2 c.c. of a 24 hours' broth culture added to 1 litre of sterile tap-water. In stoppered bottle at room temperature. Jan. 20th to Feb. 6th, 1917.

At start	43,250 per c.c.	After 14 days	absent from 0·1 c.c.*
After 3 days	37,770 „	„ 21 „	„ „ 1·0 c.c.
„ 7 „	18,800 „		

* Larger amounts not examined.

Exp. XIX. *B. coli* isolated from the fresh cow excreta used for Exp. XIII. 0·1 c.c. of a 24 hours' peptone water culture added to 1 litre sterile tap-water. In stoppered bottle at room temperature. Jan. to April, 1917.

At start	11,250 per c.c.	After 5 weeks	560,000 per c.c.
After 3 days	8250 „	„ 6 „	548,000 „
„ 7 „	4500 „	„ 7 „	456,000 „
„ 2 weeks	6500 „	„ 8 „	460,000 „
„ 3 „	43,000 „	„ 9 „	510,000 „
„ 4 „	95,000 „	„ 11 „	565,000 „

Experiment not continued further. The organism was one of the "high ratio" (CO_2 : H) type described by Clark and Lubbs, produced a definite capsule in milk, fermented lactose and saccharose well, especially the latter, and produced indol in small amount.

Exp. XXII. An experiment to test the relative viability of capsulated and non-capsulated *B. coli* in unsterilised tap-water.

One strain of each type was used and 0·03 c.c. of a 24 hours' peptone water culture was added to 1 litre of tap-water. A very hard water free from *B. coli* unsterilised and kept in separate stoppered bottles at room temperature under exactly similar conditions. The enumerations were made upon lactose neutral red bile salt agar. March, April and May, 1917.

	Non-capsulated	Capsulated
At start (per c.c.)	4400	7440
After 31 days	25	440
„ 43 „	3	176
„ 50 „	0	122
„ 57 „	0	81
„ 70 „	—	30
„ 84 „	—	25
„ 98 „	—	6

The capsulated strain was still alive in the unsterilised water at the end of 14 weeks while the non-capsulated type was dead at end of 7 weeks.

Exp. XXIV. A repetition of Exp. XXII with different strains of *B. coli* and using a different water.

One strain of each type was used and 0·03 c.c. of a 24 hours' peptone water culture was added to half a litre of water. The water used was a rather hard limestone water but free from *B. coli*. The water was used unsterilised and the samples were kept under similar conditions in stoppered bottles at room temperature. Enumerations upon lactose neutral red bile salt agar. Carried out from May 11th to June 23rd, 1917.

	Non-capsulated	Capsulated
At start (per c.c.)	8000	4920
After 14 days	960	980
„ 28 „	0 (absent from 1 c.c.)	540
„ 42 „	0 „ „	29

Summary.

The experiments with individual strains in sterile water show a marked difference between the behaviour of the streptococci and *B. coli*. In all cases the streptococci diminished in numbers and in two experiments were practically eliminated at the end of 2 weeks while in the other two they did not survive very much longer. On the other hand the *B. coli* strains although in a medium which contained little or no organic matter, and which was not in any sense a nutrient medium, showed in every case multiplication, in most extensive multiplication. This was rather contrary to our anticipations as although we were prepared for prolonged survival under conditions of freedom from com peting bacteria we did not anticipate such marked increase in numbers. In Exp. XIX the bacillus tested was still present over fifty-fold of the original number at the end of 11 weeks.

We have given some attention to the characters of the surviving types of *B. coli* in the tank experiments. Generally speaking they were quite normal in respect of fermentation reactions but on three occasions, Exps. XIII, XV and XX, the capsulated "high ratio" (CO_2 : H) coli of Clark and Lubbs were found, and in Exps. IV, XX and XXI the surviving organisms isolated were also capsulated but not "high ratio" organisms. It appears possible that the capacity to form capsules has something to do with the viability of these organisms and Exps. XXII and XXIV are evidence in this direction, but it is to be noted that the surviving *B. coli* in Exp. XVI after 7 weeks produced no definite capsule when grown in milk.

In Exp. XX the original sewage was plated out upon lactose neutral

red bile salt agar and 10 strains of *B. coli* isolated of which 50 per cent. showed capsule formation. Fourteen days later 11 strains were isolated from the sewage tank water and of these 90 per cent. were weak lactose fermenters and showed no capsule formation. In the same experiment 2 strains isolated after 4 weeks and another after 5 weeks showed definite capsule formation. The ultimate *B. coli* survivors were apparently all capsulated organisms, although at the start as many non-capsulated as capsulated organisms were present.

The tank experiments with excreta or sewage added to a large bulk of water yielded minor differences in the individual experiments, but in general they all show a rapid diminution and elimination of the streptococci and a continuous but not quite so rapid diminution in the number of *B. coli*. With the latter it was more common to find persistence in small numbers for a period extending to many weeks. The elimination of the streptococci was particularly uniform and rapid. At the end of 2 weeks in only one experiment were they present in more than insignificant numbers.

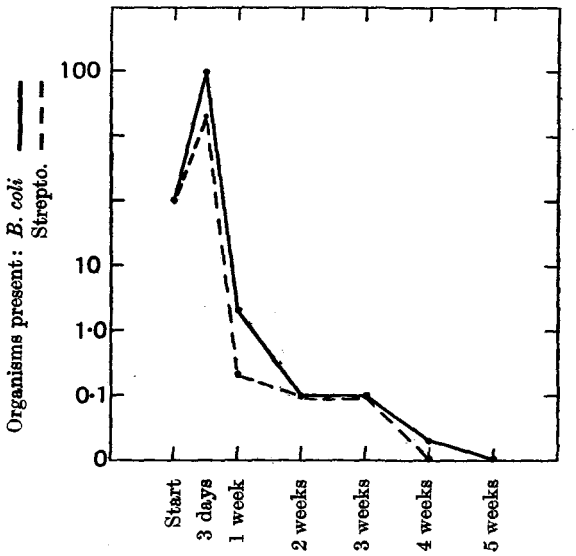
While the diminution was rather more rapid for excreta than for sewage for both streptococci and *B. coli*, no definite constant differences in relation to the kind of contamination could be made out.

The decline curves of both organisms agree very closely as can be most readily seen if the figures are plotted out as graphs. Three of the experiments, in which the initial numbers of *B. coli* and streptococci were identical, and also Exp. XI are set out in this way.

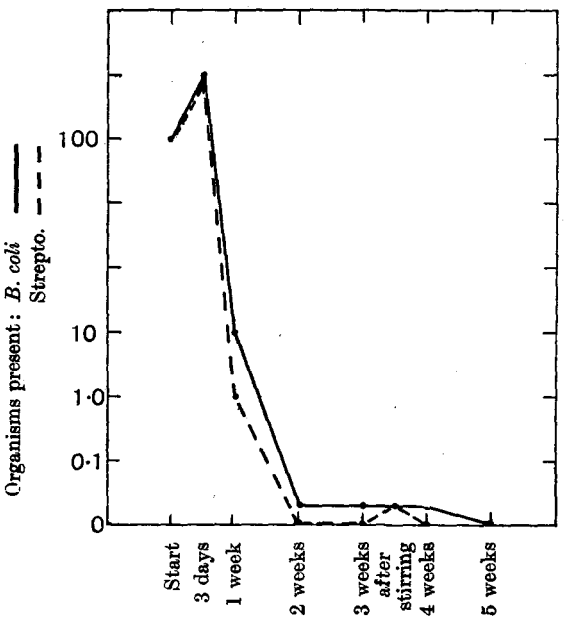
The available data is hardly extensive enough to enable deductions to be drawn other than broad and general ones, but the facts add confirmation to the view that the presence of either streptococci or *B. coli* in considerable numbers, *i.e.* in 1 or even 10 c.c. of a water, can only indicate contamination considerable in amount and of recent origin.

In particular the finding of streptococci in any numbers can be accepted as indicating considerable and recent contamination. We consider that the streptococcus determination is very valuable on its positive side as an indication of recent contamination. As a means of judging of the recency of the contamination it is even more valuable than the *B. coli* enumeration.

Put another way our experimental data shows that in nearly every case there is a marked diminution in the number of both *B. coli* and streptococci at the end of even 1 week, so that it follows that when these organisms are found in large numbers the contamination must have been either very recent or especially abundant.



EXP. III. *Domestic Sewage.* April 17th—May 22nd, 1916.



EXP. X. *Human Excreta* (Paratyphoid Convalescent).
Nov. 6th—Dec. 11th, 1916.

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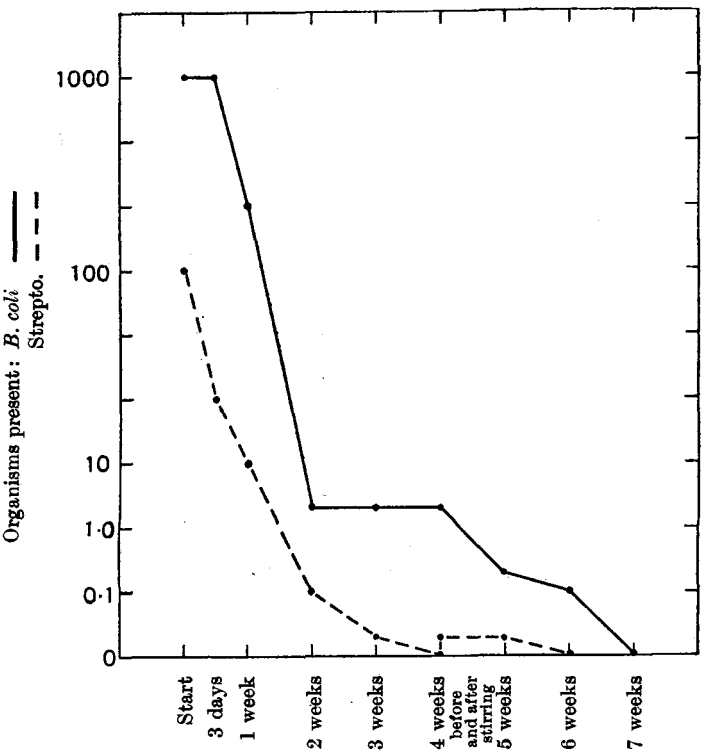
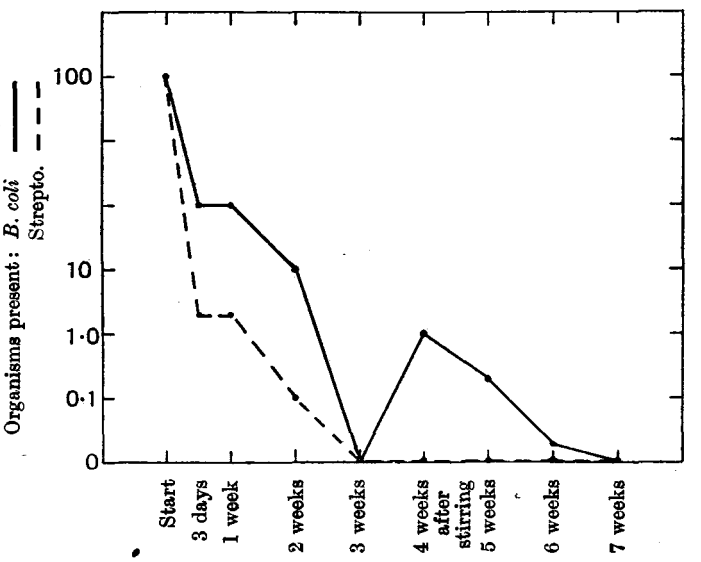


EXP. XI. *Human Excreta* (Paratyphoid Convalescent). Nov. 17th, 1916—Jan. 5th, 1917.



EXP. XIII. *Cow Manure.* Dec. 12th, 1916—Jan. 23rd, 1917.

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