THE RELATIVE ABUNDANCE OF BACILLUS COLI COMMUNIS IN RIVER WATER AS AN INDEX OF THE SELF-PURIFICATION OF STREAMS.

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It is well known that fresh sewage always contains large numbers of the common colon bacillus. It is also true that when a water source is polluted with any considerable quantity of fresh sewage it is usually possible to demonstrate the presence of the colon bacillus in such water. Upon these familiar facts have been based various methods and conclusions of greater or less value to public hygiene.

The particular method of gauging the so-called self-purification of a stream by the relative abundance of *B. coli communis* at different points is not a new one, but, so far as the writer is aware, it has not been often applied on a large scale.

Theobald Smith's ingenious method of estimating the approximate number of faecal bacteria in water by the fermentation tube was first used in the study of the self-purification problem by Smith and Brown(8). The work of these authors was carried out upon the Mohawk and Hudson Rivers under the auspices of the New York State Board of Health, and aided materially in the solution of local problems. Owing, however, to the relatively low degree of pollution obtaining in these particular river waters, as is shown both by the chlorine determinations and by the small number of faecal bacteria, the contrast between different points along the course of the river is not very marked.

Some investigations also have been carried out by Hammerl(4) upon the river Mur above and below Gratz, but the number of this author's determinations, as recorded in his article, are so few, and his method[1]

[1] Suspicious colonies upon a gelatin plate were fished and tested "for ability to grow at blood temperature, to curdle milk and to produce gas in media containing sugar," *op. cit.*, p. 537.

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for the detection and enumeration of colon bacilli is so inadequate that not much weight can be attached to his conclusions.

From a general standpoint it is clear that the question of self-purification can be most advantageously studied where the proportion

of sewage added to river water is very high, so that slight fluctuations due to temporary and local conditions, to incomplete mingling and to other minor factors, are wholly submerged by a gross and constant

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pollution. A particularly favourable opportunity has been recently afforded the writer in the course of an investigation of the conditions created in the Illinois Valley by the discharge of the sewage of the city of Chicago into the Desplaines River. This stream unites with the Kankakee River, nineteen miles below the point where the Chicago sewage is received, to form the Illinois, a stream flowing 268 miles to the Mississippi (see map, p. 296). The enormous initial pollution and the fact that during certain seasons of the year the dilution from rainfall and run-off is slight[1] render it a comparatively easy task to trace the progressive purification in the flowing stream. Few other rivers, in which the process of self-purification has been studied, present so fortunate a union of three important conditions: extreme pollution, relatively little dilution, and great length.

During the studies of the Illinois River upon which the writer has been recently engaged in behalf of the Sanitary District of Chicago he has had occasion to determine the relative number of faecal bacteria in the river water at different points. The methods employed and the results obtained are presented here through the courtesy of Dr Arthur R. Reynolds, Director of the Streams Examination[2].

[1] A chlorine content as high as 40 (parts per million) has been found at the mouth of the Illinois.

[2] For the sake of clearness the local conditions leading to the inquiry may be briefly summarized. (For a fuller description see (6).) A large part of the sewage of the city of Chicago has for many years flowed into the Chicago River, which being of small volume has become practically an open sewer. To prevent pollution of the water supply by the discharge of this sewage into Lake Michigan a pumping-station at the union of the South Branch of the river with the Illinois and Michigan Canal (Bridgeport, see map) has been utilized for pumping the river water into the canal. The amount so discharged has been estimated at about 35,000 cubic feet per minute. The canal unites with the Desplaines River a little distance above Joliet (see map), and by this route a large part of the sewage of Chicago has made its way down the Illinois Valley. As is seen by the map the canal is continued to La Salle, from which point the Illinois River is navigable to its mouth. As might be supposed, however, comparatively little sewage passes by this circuitous route. The large and expensive Sanitary Canal, which has been just completed, is designed to carry off the sewage by gravity flow, at the same time greatly diluting it with the purer water of Lake Michigan, and providing for the disposal of the sewage of the whole district. By this means the current in the Chicago River is permanently reversed, and all danger of pollution of the water supply from this source effectually prevented. The Sanitary Canal (not shown on map) runs nearly parallel to the old Illinois and Michigan Canal as far as Lockport, and discharges into the Desplaines River bed at this point. The flow prescribed by law is 300,000 cubic feet per minute for the present population of Chicago. The controlling gates of the Sanitary Canal at Lockport were first opened on January 17th, 1900.

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Methods.

In the beginning of the work use was made of the method of direct inoculation of water into the fermentation tube as suggested by Smith in 1893, but this procedure was soon abandoned in favour of another method which was continued through the major part of the investigation. This consisted in a preliminary incubation of a measured quantity of water in carbol-broth. The carbol-broth was prepared by adding 1 c.c. of a 1 % solution of carbolic acid in sterile water to tubes containing 9 c.c. of sterile broth of the standard composition. The use of measured quantities of fluid in this way of course necessitates allowance for evaporation during sterilization. By careful attention to the size of the tube and to the period of sterilization in the Arnold steam sterilizer, it has been found possible to calculate very closely the loss of fluid during heating and to make due allowance for it; subsequent evaporation before use has been guarded against. The carbol-broth which we have used has been first rendered neutral to phenolphthalein and then acidified by the addition of 5·5 c.c. of normal acid per litre.

In carrying out the method, 1 c.c. of a suitable dilution of the water has been added to a tube of carbol-broth and incubated at 38° C. for 18—24 hours. Platings from this broth have then been made in litmus-lactose-agar (5 c.c. normal alkali per litre). If red colonies developed on the medium at 38° C. they were transferred to tubes and tested at once for (1) Gas-production in dextrose-broth in the fermentation tube; (2) Indol-production in sugar-free broth; (3) Coagulation of milk; (4) Liquefaction of gelatin.

During a part of the investigation another method was employed consisting of the introduction of water directly into dextrose-broth fermentation tubes without preliminary incubation. The dextrose-broth was prepared with fresh meat from which the muscle-sugar had been removed by Smith's method, and to this sugar-free broth 1 % of dextrose was subsequently added. The broth was made neutral to phenolphthalein. After inoculation with the water the tubes were incubated at 38° C. for 48 hours, gas readings being taken at 24-hour intervals. At the end of 48 hours all tubes showing the formation of gas were removed from the incubator, cooled to the room temperature and the absorption of CO_2 determined by the addition of a 2 % solution of NaOH. It has been found necessary to take precautions against

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incomplete absorption of the CO_2, especially where large tubes are used and the amount of gas formed is considerable.

The use of litmus-lactose-agar for plating water direct proved entirely unadapted to the conditions of our work, and frequently failed to reveal the presence of the colon bacillus when the two methods above mentioned showed conclusively that this bacillus was present.

Although many other methods were experimented with and carefully compared during the course of the work, only findings obtained by the carbol-broth and dextrose fermentation methods are included in the following tables. A comparison of the two methods [5] showed that while in the main the results tally closely, the carbol-broth method is in general to be preferred for highly polluted waters, while for relatively pure waters the use of the fermentation tube direct appears to be a slightly more delicate test.

The interpretation of the results obtained by the respective methods demands some explanation, since the whole inquiry hinges upon the meaning of the records. It may be said at the outset that no attempt is made in this paper to record separately the occurrence of various members of the colon group of organisms, or to pass judgement upon the sanitary significance attaching to the presence of various kinds of colon and paracolon bacilli. For reasons easily understood such a subdivision of material would be entirely foreign to the immediate problem. A general and arbitrary standard has of necessity been chosen. In the present state of uncertainty among bacteriologists regarding classification within the colon group, I have thought it best for the purposes of this investigation to adopt a somewhat comprehensive grouping and to include among "colon bacilli" (or "faecal bacteria") certain colon-like organisms showing a fundamental biological relationship. It should perhaps be expressly stated that the term "colon bacillus" is employed in this paper in this general sense and is not used to designate a sharply defined single "species."

The carbol-broth method, as described above, has given results variously designated in the tables as +, – or ?. The sign +, as here used, indicates that colonies have been isolated which gave the typical characters of *B. coli* in (1) Fermentation tube (dextrose-broth); (2) Sugar-free broth for indol; (3) Milk; (4) Gelatin.

The sign – is used to denote those cases where, upon plating in litmus-lactose-agar, after incubation in the carbol-broth, careful search failed to reveal any red colonies; under this head also are placed those cases, not very numerous, where pure cultures obtained from a red

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colony have failed to yield an excess of H in the fermentation tube[1].

In the doubtful class are included those instances sometimes encountered, where the organism isolated from a red colony produces the typical mixture of gases in the fermentation tube, but fails to respond positively to one or two of the other characteristic biological tests, (2) being the determination most frequently at variance.

The results obtained by the dextrose-broth method are tabulated on the following basis: The sign + is used for those inoculations yielding a total gas production of more than 20 % of the tube length and showing on absorption an appreciable excess of H. If pure cultures are isolated from such tubes organisms possessed of the biological characters above cited will almost invariably be found. There is invariably a mixture of different kinds of organisms in these tubes, and it is sometimes necessary to examine a great many colonies. When this has been done we have never failed to find the colon bacillus. The error involved in the assumption that the colon bacillus is always present in these cases is probably small. The close agreement of the results obtained by this method with those reached by the carbol-broth method, which appears on the face to be more rigorous, lends further countenance to this view.

Under the sign – are included those determinations in which no gas or only a small amount of gas—less than 10 %—was produced. There is perhaps a larger measure of uncertainty regarding the determinations classified under this head; it is possible that the colon bacillus was present in some instances where no gas or only a slight amount was formed, but these cases must have been rare since we have never been able to isolate the colon bacillus on gelatin or litmus-lactose-agar plates made from such tubes. An exception must of course be made to this statement in cases where sewage or highly polluted water has been inoculated into the fermentation tube without proper dilution, since in such cases it sometimes occurs that only a small amount of gas collects in 48 hours, the colon bacillus being apparently overgrown by other sewage bacteria.

In the doubtful class are placed those determinations giving a total gas production of 10—20 %, and those with a total gas production of more than 20 % and absorption-test showing an appreciable excess of

[1] The current assumption that the gas remaining after absorption with caustic potash is H is here followed, although it is probable that other gases, such as N and CH_4, are present in small quantities.

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CO_2. The majority of the determinations so classified might fairly be regarded as negative and I believe that only a slight error would be incurred if this were done. I have preferred, however, to adopt the more unequivocal arrangement.

The samples of water examined in this work were collected and shipped to the laboratory in the way I have elsewhere described [6]. The effect of the ice-packing upon the number of colonies appearing in the ordinary plate count has been already discussed [7]. There is need, however, to consider briefly in this place the influence of the low temperature upon the number of colon bacilli. Freudenreich [2] has called attention to the fact that in both sterilized and unsterilized waters maintained at room temperature after inoculation with *B. coli* there is sometimes an increase and sometimes a decrease. From this he draws the conclusion that if water samples cannot be examined for the colon bacillus immediately after collection they should be packed in ice for transportation to the laboratory. The hasty assumption that no change in the colon content occurs in ice-packed samples obviously needs justification. Our own experiments are too few in number to warrant generalization, but so far as they go they indicate that no material change occurs in ice-packed samples within 48 hours, a period longer than that usually consumed in transportation.

These experiments may be briefly summarized. In five examinations of various natural waters in different dilutions no change was observed in the number of colon bacilli after the water had remained packed in ice for 46—48 hours. In one other examination there was no appreciable change in 24 hours, but a slight decrease was observable after 48 hours. In another examination the colon bacillus was found in 1 c.c. (2 determinations) at the outset, while it was not in 0·1 c.c.; eight days later the proportion was the same: it was present in 1 c.c. (2 tubes), but not in 0·1 c.c. In another ice-packed sample the colon bacillus was found in 0·1 c.c. immediately after collection, and again after the lapse of 24 hours, but not after six days. One typical experiment of this sort may be tabulated here:

	0·1 c.c.	1 c.c.	2 c.c.
Immediately after collection	0 0	+ + + 0	
After being packed in ice for 47 hours, temp. 1·5° to 3° C.	0 0 0 0	+ + + +	+ + 0 0

Examinations of three separate water samples allowed to stand at room temperature showed neither increase nor decrease after 46—48 hours.

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Taken as a whole our observations clearly indicate that the changes in the colon content of a water which occur on standing are not very rapid and are usually in the direction of a diminution. It may be fairly assumed that the transportation error is less for the colon determinations than for the colony count.

Together with the detailed tables, a few auxiliary data may be presented. I have not been able to discover any statement by authors concerning the number of colon bacilli normally found in fresh sewage and therefore desire to incorporate here a few observations made in my laboratory by Mr W. G. Sackett. Samples of sewage collected from the 56th St. sewer and composed almost entirely of house-sewage were examined immediately after collection with the following results:

Fresh Sewage[1].

Date	·00001 c.c.			·0001 c.c.			Chlorine
1900	+	−	?	+	−	?	(parts per million)
May 16	2	0	0		—		—
Nov. 16	0	5	0		—		66
20	2	2	1		—		—
21	0	5	0		—		—
24		—		3	1	1	70
27		—		1	3	1	70
30	1	4	0	3	0	2	73
Dec. 4	0	5	0	4	1	0	68
5	3	2	0	4	0	1	62
11	2	3	0	5	0	0	68
12	2	0	3	5	0	0	68
14	3	1	1	5	0	0	66

TABLE I. Illinois and Michigan Canal, Lockport.

Serial number	Date	·00001 c.c.			·0001 c.c.			·001 c.c.			·01 c.c.		·1 c.c.	
	1899	+	−	?	+	−	?	+	−	?	+	−	+	−
526	Aug. 29										1	0	1	0
876	Oct. 31					4	0	2	0					
914	Nov. 7	0	4		1	0	1							
950	14	2	2		1	0	1							
982	21	1	1		4	0								
1011	28	0	2		2	1	1							
1042	Dec. 5	0	0	1	1	0	1							
1072	12	0	2		2	1	1							
1110	19	0	2		3	0	1							
1146	28	0	2		4	0								

[1] Collected from the 56th St. sewer between 3 and 4 p.m. and examined within an hour after collection.

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Serial number	Date 1900	·00001 c.c.			·0001 c.c.			·001 c.c.			·01 c.c.		·1 c.c.	
		+	−	?	+	−	?	+	−	?	+	−	+	−
1168	Jan. 3	0	2		2	2								
1199	9	0	2		3	1		2	0					
1239	16	1	0		2	0								
1269	23	0	4		4	0								
1303	30	0	4		1	3								
1334	Feb. 6	0	2		2	2								
1457	Mar. 6	0	4		1	3								
1493	13	0	2		2	1	1	1	0					
1522	20				1	2		1	2					
1563	28	1	2		3	0								
1597	April 3	0	3		1	2								
1636	10	1	1		2	0								
1675	17	0	2		0	1	1	1	0					
1717	24	1	0		1	1		0	0	1				
1753	May 2	0	1		0	2		1	0		1	0		
1786	8	0	1		1	0		2	0		1	0		
1817	15	0	1		1	0		1	0					
1856	22	1	1		1	0	1							
1895	29	0	2		1	1								
1923	June 5	0	2		1	0	1							
1959	12	0	2		2	0								
2000	19				0	1		0	1		1	0	1	
2032	26				0	2		0	2					

TABLE II. Desplaines River, Lockport.

Serial number	Date 1899	·01 c.c.			·1 c.c.			1 c.c.		
		+	−	?	+	−	?	+	−	?
383	Aug. 1				0	1		0	1	
418	8				0	1		1	0	
498	22				0	1				
527	29				0	1		1	0	
877	Oct. 31				1	0				
915	Nov. 7				0	0	1	0	0	1
951	14				0	1		0	0	1
1012	28	0	3		0	2				

TABLE III. Kankakee River, Wilmington.

Serial number	Date 1899	·1 c.c.			1 c.c.		
		+	−	?	+	−	?
415	Aug. 7	0	1		1	0	
496	22	1	0				
528	29	1	0		1	0	
566	Sept. 4	0	1		1	0	
875	Oct. 30	1	0				
913	Nov. 6	0	0	1	0	1	
981	20				1	0	

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TABLE IV. Illinois River, Morris.

Serial number	Date	·0001 c.c.			·001 c.c.			·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	–	?	+	–	?	+	–	?	+	–	?	+	–	?
879	Oct. 31				2	1		2	0							
917	Nov. 7				2	1	1	2	0							
962	15	0	2		3	0		3	0	1						
985	21	1	2		2	3		3	0							
1014	28	0	1		4	0		4	0							
	1900															
1196	Jan. 7				1	0	1	3	0	1	2	0				
1209	10				1	0		1	0							
1248	19				1	0		2	0							
1296	27				1	2		2	0		2	0				
1307	30				0	2		2	2		2	0				
1338	Feb. 6							0	4		2	0				
1373	13				0	2		1	3		2	0				
1400	20				0	2		1	2	1	4	0				
1461	Mar. 8				1	1		3	1		2	0				
1500	15				0	1		1	1	1	1	1				
1526	20				0	2		1	0	1	2	0				
1571	29				0	1		1	1		2	0				
1602	April 4				0	1		1	1		0	1				
1640	10				1	0		2	0		1	0				
1680	17				0	1		2	0		1	0				
1715	23				0	1		0	1	1	1	0				
1758	May 2							0	1		0	1	1	1	0	
1784	8							0	1		2	0		1	0	
1822	16							0	1		1	1		1	0	
1854	22							0	1		0	2		1	0	
1893	29							0	1		1	1		2	0	
1928	June 5							1	0		2	0		0	1	
1962	12							0	1		2	0		1	0	
1996	19							0	1		2	0		1	0	
2037	27							0	1		2	0		1	0	

TABLE V. Fox River, Ottawa.

Serial number	Date	·1 c.c.			1 c.c.			5 c.c.		
	1899	+	–	?	+	–	?	+	–	?
384	Aug. 1	0	1		0	1				
420	8	0	1		0	1				
500	22	0	1							
529	29	0	1		0	0	1			
881	Oct. 31	0	1		1	0				
918	Nov. 7	0	1		0	1				
953	14	0	1		0	1				
986	21	0	1		0	1				
1015	28	0	1		0	1				

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Serial number	Date	·1 c.c.			1 c.c.			5 c.c.		
	1900	+	−	?	+	−	?	+	−	?
1282	Jan. 26	0	3	1	1	2				
1528	Mar. 21	0	3		0	3				
1568	28				0	2	1	2	0	
1603	April 4	1	0		2	0		1	0	
1642	11				2	0		1	0	
1681	18	0	1		0	2		1	0	
1722	25	0	1		0	1	1	0	0	1
1759	May 2	0	1		0	2		1	0	
1790	9	1	0		2	0		1	0	
1823	16	0	1		0	2		1	0	
1859	23	0	1		0	2		0	1	
1930	June 6	0	1		0	2		0	0	1
1963	12	0	1		0	1	1	1	0	
1997	19	0	1		0	2		1	0	
2035	26	0	1		1	1		1	0	

TABLE VI. Illinois River, Ottawa.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
	1899	+	−	?	+	−	?	+	−	?	+	−	?
385	Aug. 1				0	1		0	1				
421	8				0	1		1	0				
501	22				0	1							
530	29				0	1		0	0	1			
880	Oct. 31	0	4		1	0	2						
919	Nov. 7	0	2		1	2	1	1	0	1			
954	14	0	2		2	2		2	0				
987	21	1	1		1	0	3	2	0				
1016	28	0	2		0	3	1	0	0	2			
1076	Dec. 13	1	1		3	1		2	0				
1130	22	1	0		1	0		1	0				
1153	29	1	0	1	1	0	3	0	0	2			
	1900												
1174	Jan. 5	1	0		3	0	1	2	0				
1221	12	0	1		2	0		1	0				
1250	19	0	2		3	0	1	2	0				
1283	26	0	0	2	4	0		1	0				
1340	Feb. 6				1	3		2	0				
1402	21				0	2		3	1				
1455	Mar. 6				0	4		0	19	1			
1490	13	0	2		2	2		2	0				
1529	21	0	1	1	2	0		1	1				
1569	28	0	2		1	1		1	1				
1604	April 4	0	1		0	2		1	0				
1643	11	0	1		0	2		0	1				
1682	18				1	1		0	0	2			

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TABLE VI. Illinois River, Ottawa (*continued*).

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
	1900	+	−	?	+	−	?	+	−	?	+	−	?
1723	April 25				0	2		0	2				
1760	May 2	0	1		0	2		0	0	1			
1791	9				1	0		2	0				
1824	16				1	0		0	0	2	1	0	
1860	23				0	0	1	0	0	2	0	0	1
1931	June 6	0	1		0	1		0	0	2	0	0	1
1964	12	1	0		0	0	1	2	0		1	0	
1998	19	0	1		1	0		1	0		1	0	
2036	26	0	1		0	1		1	1		1	0	

TABLE VII. Big Vermilion River, La Salle.

Serial number	Date	·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?
388	Aug. 2				0	1	
423	9				0	0	1
502	23	0	1		0	0	1
533	30	0	1		0	1	
882	Nov. 1	1	0		1	0	
920	8				0	1	
959	15				0	1	
988	22	0	1		1	0	
1017	28	0	1		1	0	

TABLE VIII. Illinois River, La Salle.

Serial number	Date	·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?
389	Aug. 2				0	0	1
424	9	1	0		0	0	1
503	23	0	1		0	0	1
534	30	0	1		0	0	1
883	Nov. 1	1	0		1	0	
960	15	0	1		1	0	
989	22	0	1		0	0	1
1018	28	0	1		1	0	

TABLE IX. Illinois River, Henry.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?	+	−	?
107	June 7							0	1	
134	14							0	1	
173	21				0	1		0	1	

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Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?	+	−	?
212	June 28				0	1		0	1	
583	Sept. 7				0	0	1	0	0	1
616	13				1	0		1	0	
652	20				1	0		1	0	
690	27	0	1		0	1				
1116	Dec. 20							4	0	
	1900									
1170	Jan. 4				1	1		1	0	1
1207	10							2	2	
1246	17				1	3		3	1	

TABLE X. Illinois River, Averyville.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?	+	−	?
119	June 9							0	1	
135	12							0	1	
174	21				0	1		0	1	
213	28							0	1	
387	Aug. 2							1	0	
580	Sept. 6				1	0		1	0	
617	13				0	1		0	1	
653	20				0	1		0	1	
691	27				0	1		0	1	
	1900									
1275	Jan. 24	0	4		0	2				
1312	31				0	2		2	2	
1344	Feb. 7				0	2		0	1	3
1380	14				0	0	2	3	0	1
1406	21				0	1	1	4	0	
1437	28				1	1		2	2	
1459	Mar. 7				0	2		2	2	
1498	14				1	0	1	0	2	1
1533	21				0	1	2	1	0	2
1570	28				0	2	1	3	0	
1608	April 4				0	1		1	0	1
1647	11				0	0	1	0	0	2
1687	18				0	2		0	2	
1727	25				0	1		0	1	1
1765	May 2				0	1		0	1	1
1795	9				0	1		0	1	1
1832	17				0	0	1	1	0	1
1864	23				0	1		0	0	2
1901	29				1	1		2	0	
1935	June 6				0	1		0	1	1
1969	13				0	1		0	2	
2006	20				0	1		2	0	
2041	27				0	1		0	2	

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TABLE XI. Illinois River, Wesley City.

Serial number	Date	·001 c.c.			·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	–	?	+	–	?	+	–	?	+	–	?
120	June 9							0	1		0	1	
136	14							0	1		0	1	
177	21							1	0		1	0	
214	28							1	0		1	0	
581	Sept. 6				0	0	1	1	0				
618	13				0	0	1	0	0	1			
692	27	0	1		0	1		0	0	1			
	1900												
1345	Feb. 7				0	2		1	3		0	0	2
1381	14				0	2		1	2	1	2	0	
1411	23				0	2		1	3		2	0	
1460	Mar. 7				0	2		1	1	2	1	1	
1534	22				0	2		2	0		2	0	
1581	30				0	2		0	1		1	1	
1610	April 5				0	1		0	2		1	0	
1648	11				0	0	1	0	2		0	0	1
1689	18				0	1		0	2				
1728	25				0	1		0	0	1	1	1	
1767	May 3				0	1		0	2		0	0	1
1796	9				0	1		1	0	1	1	0	
1829	16				1	0		2	0		1	0	
1866	23	0	1		0	1		1	0				
1902	31	1	0		1	0	1	1	0				
1938	June 7	0	1		0	1		0	2		1	0	
1973	14	0	1		0	1		0	2		1	0	
2008	21	0	1		1	1		1	0				
2042	28	0	1		0	2		0	1				

TABLE XII. Illinois River, Havana.

Serial number	Date	·1 c.c.			1 c.c.		
	1899	+	–	?	+	–	?
115	June 7				1	0	
138	14				1	0	
178	21				1	0	
221	29				1	0	
589	Sept. 7	1	0		1	0	
625	14	0	1		0	0	1
659	21	1	0		1	0	
699	29	0	1		0	1	

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TABLE XIII. Sangamon River, Chandlerville.

Serial number	Date	·01 c.c. +	·01 c.c. −	·01 c.c. ?	·1 c.c. +	·1 c.c. −	·1 c.c. ?	1 c.c. +	1 c.c. −	1 c.c. ?
	1899									
116	June 8							0	1	
182	22							1	0	
626	Sept. 14				0	0	1	0	0	1
660	21				1	0		1	0	
715	29				1	0		1	0	
	1900									
1276	Jan. 25				1	3		4	0	
1315	Feb. 1				0	4		0	4	
1355	8				2	0		4	0	
1384	15				2	1	1	1	0	1
1438	Mar. 2				0	1	3	3	1	
1470	8				3	1		2	0	2
1508	15				1	1	1	0	3	
1544	22				3	0		3	0	
1572	29	0	2		0	2		0	1	1
1613	April 5	0	2		1	1		1	1	
1652	12	0	2		0	0	2	2	0	
1692	19	0	1		0	1		2	0	
1731	26	0	2		0	1	1	0	0	2
1770	May 3	0	1		2	0		2	0	
1805	10	1	0		2	0		1	0	
1839	17	1	0		1	0		2	0	
1875	24	1	0	1	2	0		2	0	
1911	31				1	0		1	0	
1945	June 7	0	2		0	2		1	0	
1981	14	0	1		0	2		1	0	
2011	21	2	0		0	1	1	2	0	
2051	28	0	2		0	1	1	1	0	

TABLE XIV. Illinois River, Grafton.

Serial number	Date	·01 c.c. +	·01 c.c. −	·01 c.c. ?	·1 c.c. +	·1 c.c. −	·1 c.c. ?	1 c.c. +	1 c.c. −	1 c.c. ?	5 c.c. +	5 c.c. −	5 c.c. ?
	1899												
117	June 8							0	1				
184	22							0	1				
222	30							0	1				
591	Sept. 7				0	1		0	1				
628	14				1	0		1	0				
662	21				1	0		1	0				
701	29				0	1		1	0				
996	Nov. 23	1	1		2	0		2	0				
1025	Dec. 1	0	1		1	0		1	0				
1057	6	0	1		0	1		1	0				

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Table XIV. Illinois River, Grafton (*continued*).

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
	1899	+	−	?	+	−	?	+	−	?	+	−	?
1098	Dec. 15				0	1		0	1	1			
1127	22				0	0	1	1	0	1			
1156	29				0	1		0	0	2			
	1900												
1187	Jan. 5				0	1		0	2				
1217	10				0	1		0	1	1			
1261	19				0	4		0	2	2			
1287	26				1	1		2	1	1			
1317	Feb. 1	0	2		0	2		2	2				
1356	8				0	1	1	2	1				
1386	15				1	0	1	2	0	2			
1408	22				0	2		3	0	1			
1484	Mar. 9				1	1		4	0				
1509	16				2	0		4	0				
1536	22				0	3		0	1	2			
1579	29				0	3		2	0	1			
1615	April 5				2	0		1	0	1			
1654	12				0	1		1	0	1			
1693	19				0	1		1	0	1			
1732	26				0	1		1	1				
1771	May 3				1	0		2	0				
1806	10				1	0		2	0				
1841	17				1	0		2	0				
1877	24				1	0		2	0				
1912	31				0	1		0	2				
1946	June 8				0	1		0	0	1	0	0	1
1983	15				0	1		1	0		1	0	
2019	22				0	1		2	0		0	0	1
2053	29				1	0		2	0		1	0	

Table XV. Mississippi River, Grafton.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
	1899	+	−	?	+	−	?	+	−	?	+	−	?
118	June 8							0	1				
185	22				0	1							
223	30							1	0				
592	Sept. 7				0	1		0	1				
629	14				0	1		0	0	1			
663	21				1	0		1	0				
702	29				0	1		1	0				
1026	Dec. 1	0	1		0	1		1	0				
1058	6	0	1		0	1		0	0	1			
1099	15				0	1		1	1				
1128	22				0	1		1	0	1			
1157	29				0	1		0	2				

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Serial number	Date 1900	·01 c.c. +	·01 c.c. −	·01 c.c. ?	·1 c.c. +	·1 c.c. −	·1 c.c. ?	1 c.c. +	1 c.c. −	1 c.c. ?	5 c.c. +	5 c.c. −	5 c.c. ?
1188	Jan. 5				0	1		1	1				
1218	10				0	1		1	1				
1262	19				0	4		1	2	1			
1286	26				1	1		3	1				
1318	Feb. 1				0	2		0	2	2			
1357	8				1	1		1	1	2			
1387	15				1	1		3	1				
1409	22				0	2		2	0	2			
1485	Mar. 9				0	2		2	1				
1510	16				1	1		3	0	1			
1537	22				1	1	1	3	0				
1580	29				0	3		1	2				
1616	April 5				1	1		0	1	1			
1655	12				0	2		1	1				
1694	19				0	1		0	2				
1733	26				0	1		3	0				
1772	May 3				0	1		1	0	1			
1807	10				1	0		2	0				
1878	24				1	0		1	0	1			
1913	31				1	0		0	0	1			
1947	June 8				0	0	1	0	0	1	1	0	
1984	14				0	1		0	0	1	1	0	
2019	22				0	1		0	2		0	1	
2054	29				0	1		2	0		1	0	

Table XVI. Mississippi River, Cross-Section at Alton.

	Serial number	Date 1899	·1 c.c. +	·1 c.c. −	·1 c.c. ?	1 c.c. +	1 c.c. −	1 c.c. ?
East Bank	248	July 6	0	1		0	1	
	284	13	0	1		0	0	1
	319	20	0	1		0	1	
	356	27	0	1		0	1	
	736	Oct. 5	0	1		0	1	
	773	12	0	1		1	0	
	846	26	0	1		0	1	
East Centre	249	July 6	0	1		0	1	
	285	13	0	0	1	0	0	1
	320	20	0	1		0	1	
	357	27	1	0		1	0	
	737	Oct. 5	0	1		0	1	
	774	12	0	1		0	0	1
	847	26	0	1		0	1	

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TABLE XVI. Mississippi River, Cross-Section at Alton (*continued*).

	Serial number	Date	·1 c.c.			1 c.c.		
		1899	+	−	?	+	−	?
Centre	250	July 6	0	1		0	1	
	286	13	0	1		1	0	
	321	20	0	1		0	1	
	358	27	0	1		0	1	
	738	Oct. 5	0	1		0	1	
	775	12	0	1		0	1	
	848	26	1	0		0	0	1
West Centre	251	July 6	0	1		1	0	
	287	13	1	0		0	1	
	322	20	0	1		0	1	
	359	27	0	1		0	1	
	739	Oct. 5	0	1		0	1	
	776	12	0	1		0	0	1
	849	26	1	0		1	0	
West Bank	252	July 6	0	1		0	1	
	288	13	0	1		0	1	
	323	20	0	1		1	0	
	360	27	0	1		1	0	
	740	Oct. 5	0	1		0	1	

TABLE XVII. Missouri River, West Alton.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
	1899	+	−	?	+	−	?	+	−	?
376	July 28				0	1		0	1	
671	Sept. 23				0	1		0	0	1
714	29				0	0	1	0	0	1
749	Oct. 6				0	1		1	0	
789	13				0	1		0	1	
869	27				0	1		0	1	
	1900									
1189	Jan. 5				0	2		0	4	
1235	10				0	1		1	0	
1263	19				1	3		1	2	1
1295	26				0	2		3	1	
1320	Feb. 2				0	2		0	4	
1358	8				0	2		2	2	
1388	15				0	2		0	3	1
1410	22				0	2		0	4	
1439	Mar. 2				0	4		4	4	
1471	8				0	2		2	1	1
1499	14				2	0		0	1	2

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Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.		
	1900	+	−	?	+	−	?	+	−	?
1556	Mar. 23				0	0	1	2	0	
1594	30				2	0		2	0	
1609	April 4				2	0		2	0	
1650	11				1	0		2	0	
1690	18	0	1		0	1	1	1	1	
1721	25				0	1		2	0	
1766	May 3				0	0	1	1	0	1
1798	10				1	0		1	0	
1831	16	0	1		1	0	1			
1865	23	1	0		1	0		1	0	
1910	31	0	1		2	0		1	0	
1937	June 7				2	0		2	0	
1970	13				1	0		2	0	
2007	20	1	0		2	0		1	0	
2050	28	2	0		1	0		1	0	

TABLE XVIII. Mississippi River, Intake-Tower, St Louis Water-works, Mitchell.

Serial number	Date	·01 c.c.			·1 c.c.			1 c.c.			5 c.c.		
	1899	+	−	?	+	−	?	+	−	?	+	−	?
295	July 14				1	0		1	0				
330	21				0	1		1	0				
373	28				1	0		1	0				
743	Oct. 6				0	1		0	0	1			
859	27				0	1		0	1				
	1900												
1299	Jan. 27				1	1		1	1	2			
1361	Feb. 9				1	0	1	4	0				
1426	24				1	1		1	0	3			
1474	Mar. 9				0	0	1	1	0	1			
1547	23				3	0		2	0	1			
1585	30				0	2		1	1		1	0	
1624	April 6				0	1		1	1				
1658	13				0	1		2	0				
1702	20				1	1		0	0	2			
1810	May 11				1	0		2	0		0	0	1
1845	18				0	0	1	0	0	2	0	0	1
1885	25				1	0		0	0	2			
1917	June 1				1	0		2	0				
1950	8				0	0	1	1	0	1			
1987	15				1	0		2	0				
2022	22	1	0		1	0		2	0				
2057	29	1	0		2	0		1	0				

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TABLE XIX. St Louis Tap-water.

Serial number	Date	·1 c.c. +	·1 c.c. −	·1 c.c. ?	1 c.c. +	1 c.c. −	1 c.c. ?	5 c.c. +	5 c.c. −	5 c.c. ?
	1899									
265	July 7	0	1		1	0				
298	15	0	1		1	0				
333	22	0	1		1	0				
365	28	0	1		0	0	1			
781	Oct. 13	0	1		0	1				
	1900									
1289	Jan. 26	1	1		1	2	0			
1347	Feb. 7	1	1		0	3	1			
1364	10	0	2		0	4				
1376	13	0	2		0	3	1			
1383	15	0	4		0	4		1		
1395	17	0	2		0	3	1			
1413	23	0	2		0	4		1	1	
1448	Mar. 5	0	2		0	3	1			
1476	9	0	1		0	1	1			
1514	17	0	1		2	0		1	0	
1549	23	0	3		2	0		1	0	
1587	30	0	2		1	1		1	0	
1626	April 6	0	1		0	2				
1660	13				0	2		1	0	
1704	21	1	1		1	1				
1743	27	0	1		0	1	1	1	0	
1778	May 4	0	1		1	1				
1812	11	0	1		2	0		1	0	
1847	18	0	1		0	0	2	1	0	
1879	25	1	0		0	2				
1914	June 1	0	1		1	1		1	0	
1952	8	0	0	1	1	1				
1989	15	0	1		1	1				
2025	23	1	0		1	0	1			
2060	29	0	1		1	0	1			

TABLE A*. Principal Stations on the Illinois River.

Collecting Stations	·00001 c.c. No. of days water exam'd	·00001 c.c. No. of days B. coli found	·0001 c.c. No. of days water exam'd	·0001 c.c. No. of days B. coli found	·001 c.c. No. of days water exam'd	·001 c.c. No. of days B. coli found	·01 c.c. No. of days water exam'd	·01 c.c. No. of days B. coli found	·1 c.c. No. of days water exam'd	·1 c.c. No. of days B. coli found
Ill. and Mich. Canal, Lockport	28	7	32	28	11	8	4	4	2	2
Ill. River, Morris	—	—	3	1	20	11	30	20	23	20
Ill. River, Ottawa	—	—	—	—	—	—	22	6	34	19
Ill. River, Averyville	—	—	—	—	—	—	1	0	27	4
Ill. River, Wesley City	—	—	—	—	7	1	22	3	26	13
Ill. River, Grafton	—	—	—	—	—	—	4	1	35	13

* See Note * on p. 315.

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TABLE B*. Illinois River at Averyville and Grafton compared with tributaries and with the Mississippi (Grafton) and Missouri (West Alton) Rivers.

Collecting Stations	·01 c.c.		·1 c.c.		1 c.c.		5 c.c.	
	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found	No. of days water exam'd	No. of days B. coli found
Ill. River, Averyville	1	0	27	4	31	13	—	—
Ill. River, Grafton	4	1	35	13	38	26	4	2
Miss. River, Grafton	2	0	34	10	35	23	4	3
Desplaines River	—	—	8	1	5	2	—	—
Kankakee River	—	—	6	3	5	4	—	—
Fox River	—	—	22	2	23	6	13	10
Big Vermilion River	—	—	5	1	9	3	—	—
Sangamon River	13	4	25	14	27	21	—	—
Missouri River	6	3	32	13	31	21	—	—

TABLE C*.

	·1 c.c.		1 c.c.	
Total, Illinois River (Averyville and Grafton)	62	17	69	39
Total, tributaries of Illinois River	66	21	69	36
Total, Mississippi and Missouri Rivers	66	23	66	44

* It will of course be observed that this method of summarizing the results is not altogether precise. The fact that on certain days and with certain dilutions more than one determination was made obviously implies the examination of a larger quantity of water at those times and the increased possibility of a positive finding. The tabulation of the results on the basis of the total number of determinations is, however, open to objection on other grounds and the method I have employed seemed to me on the whole to present fewer disadvantages.

General Considerations.

The tables show that the number of colon bacilli found in the Illinois and Michigan Canal at Lockport is at least no greater than the number often found in fresh sewage. During the period covered by our investigation the water in the canal at Lockport, which is to be regarded as partially decomposed sewage, almost invariably revealed

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the presence of colon bacilli when examined in a 1—10000 dilution and sometimes when in a 1—100000 dilution. The enormous number of colon bacilli thus indicated fairly represents the quality of the discharge into the Desplaines River at Lockport[1].

At Morris, about 27 miles below Lockport (see map) and 9 miles below the union of the Desplaines with the Kankakee, a peculiar condition exists. It has been shown elsewhere[(6)] that the mixing of the Kankakee and Desplaines waters at Morris is very incomplete, and that the composition of the samples collected in the middle of the river at this point depends upon extremely complex and variable factors. The chlorine analyses clearly demonstrate, however, that up to the middle of January, the time when the Sanitary Canal was opened, at least one-half of the water sample from the midstream at Morris was derived from the Illinois and Michigan Canal. Since the opening of the Sanitary Canal, on the average less than one-fifth of the water sample has come from Chicago sewers. The number of colon bacilli at this point is on the whole lower than can be accounted for by simple dilution[2]. This is particularly apparent during the later period of the investigation when the canal water constituted at least as much as one-tenth of the midstream water at Morris. During this period (Table IV., May and June) the colon bacillus was not often found in a dilution of ·01 c.c., an indication that about nine-tenths of the colon bacilli originally present in the sewage had perished or in some way disappeared before reaching this point.

On the way to Ottawa, about 24 miles below Morris, the mingling of the Kankakee and Desplaines rivers becomes much more complete and some additional dilution occurs[(6)]. The weekly sample from Ottawa has contained on the average at least one-half as much water of sewage origin as the midstream sample at Morris. The number of colon bacilli found in the water at Ottawa is, again, lower than would result from simple dilution.

At Averyville, 159 miles from Chicago and just above the city of Peoria, the number of colon bacilli in the water has remained about the same throughout the whole period of the investigation, and has

[1] The volume of water in the Desplaines River is insignificant at most seasons, and but for the discharge from the canal the river bed would be nearly dry for three-quarters of the year.

[2] It must be noted also that the sewage of Joliet (population, 29,353, according to the U. S. Census for 1900) contributes its quota of fresh colon bacilli to the river between Lockport and Morris.

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averaged quite as low as the number found in the various tributaries of the Illinois and in river waters in general (cf. Table B).

Immediately below Peoria (Wesley City, Table XI.) the number of colon bacteria is increased as the natural consequence of the influx of the sewage of Peoria. At the mouth of the Illinois River (Grafton, Table XIV.) the number of colon bacteria ranges slightly higher if anything than at Averyville, a condition that may perhaps be accounted for in part by the influence of the Sangamon River, which has had a persistently high colon content during the period covered by our analyses. The actual number of determinations is not large enough, however, to admit of much stress being laid upon the apparent divergence between Averyville and Grafton.

It is not intended in this paper to assume that no change occurs in the gas-producing power of the colon bacillus during its sojourn in the river water. All that the results prove is that those varieties of colon bacilli which happen to be revealed by the methods used and are characterized by the gas-producing qualities elsewhere described, disappear from the flowing stream. Whether this is due wholly to the death and sedimentation of this class of bacteria or in part also to a modification of individual cells with reference to their aerogenic power is a question too recondite to be discussed in the light of our present imperfect knowledge of the causes modifying gas production. It is quite possible that some colon bacilli may become so disguised by prolonged aquatic life as to be no longer recognizable by the methods used. It must be recalled, however, that it is always possible to recover colon bacilli possessed of typical gas-producing qualities from sewage and from polluted river waters that have been stored for some weeks in glass bottles in the laboratory. In one instance we have found colon bacilli yielding typical gas production in a 1—1000 dilution of sewage that had been standing in a bottle for forty-two days, and in another case we have found them six months after the sewage had been collected.

No indications have been noted that the *season of year* bears any very marked relation to the number of colon bacteria in the river waters examined. There appears, however, to be a slight increase in some rivers during the months of February and March when the streams are swollen by heavy rains and melting snow. On the supposition that the exhaustion of food-supply or the accumulation of harmful metabolic products is the primary cause of the disappearance of the colon bacilli and other sewage bacteria from flowing streams, the effect of flood-time

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is easy to understand. It is not simply that the bacteria are prevented by a more rapid current from settling out, but that they, *together with their food-supply*, are swept along over a greater distance in the same period of time. The actual length of time that the food-supply holds out may or may not be the same at high water as at low water, but the distance traversed is certainly greater in the former case. The same reasoning is applicable in case the heaping up of injurious substances as the result of bacterial metabolism is the lethal cause.

The possible influence of the opening of the Sanitary Canal upon the colon content of the Illinois River is a matter of considerable interest. The connection between the river and the canal was first broken through on Jan. 1, 1900, and although the controlling gates at Lockport were not opened until Jan. 17, the composition of the water pumped from the Chicago River into the Illinois and Michigan Canal at Bridgeport was changed immediately by the inrush of purer water from Lake Michigan. At Morris and Ottawa such slight alteration in the colon content as can be observed is in the direction of smaller numbers, as should indeed be anticipated on the ground of greater dilution. At Averyville no significant difference between the two periods can be noted. There is no evidence that the number of colon bacilli in the water at the mouth of the Illinois River, 160 miles below Averyville, was affected by the opening of the Sanitary Canal. During the period under consideration, in fact, the streams tributary to the Illinois contained about the same number of colon germs as did the Illinois River at Averyville.

For the sake of comparison it will be useful to cite here the more important observations that have been made regarding the presence of *B. coli communis* in the river waters of the United States. Smith and Brown[8] state that "The numbers of *Bacilli coli communis* found in water supplies from sources considered on inspection to be unpolluted, vary from nine to five [per cubic centimetre]. The numbers in Mississippi River water as supplied to St Louis vary from three to seven, and the numbers in Hudson River water at the intake of the Albany Water-works, vary from twenty to over ninety on different stages of the tide." It is interesting to note that if we employ the method of enumeration used by these authors, the number of colon bacilli which we have found in the Mississippi River at the intake tower of the St Louis Water-works would be stated as being on the average five per cubic centimetre (Table XVIII. column of ·1 c.c.), and the number in the water-supply as delivered to the consumer as one per cubic

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centimetre (Table XIX.), the latter figure being exactly the same as the number in the Illinois River at Averyville (Table X.).

Fuller[3] has recorded the finding of *B. coli communis* in the Ohio River water at Cincinnati between March 24 and December 29, 1898. Out of 197 determinations on different days a positive result was reached in 114 or about 58%. This is very close to the result reached for the Illinois River (Table C.), which, put into percentage form, gives 56% of positive results for the one cubic centimetre determinations. The number of colon bacilli in the Ohio River appears to have been somewhat greater during the summer than during the winter months.

Clark[1], who used, however, the less delicate plate method of enumeration, records an average of 47 colon bacilli per cubic centimetre in the Merrimack River water at the filter intake of the Lawrence City Water-works. In 1898 the range was from 20 per cubic centimetre in May and June to 92 in August and September; in 1899, from 8 in August to 140 in December.

It is perhaps needless to dwell upon the peculiar value of systematic observations regarding the presence and abundance of the colon bacillus in all studies of the pollution and self-purification of streams. The life-history of the colon bacillus in flowing water brings us closer to the real problems at issue than observations of any other nature. Under ordinary conditions the chief danger from sewage-polluted surface water in this country lies in the possible presence of the typhoid bacillus and perhaps of one or two other closely related organisms. Since there are no methods in common use that are universally applicable to the direct detection of these microbes, the fate of typhoid germs introduced into a stream or lake must be determined by inference. Now it is a far cry from the "chlorine," "free ammonia," and "nitrates" in a water to its content of typhoid germs, and the actual number of bacteria of all kinds in a water is a criterion of but slightly greater value. The colon bacillus, however, is biologically similar to the typhoid germ, and like the latter its presence in sewage is due almost wholly to human life and habitation. There is specific reason to believe that the duration of life of the colon bacillus in water is considerably greater than that of the typhoid bacillus, a belief that is in accord with what is known regarding the more robust character of the former organism. The best index we can secure at present, therefore, for gauging the probable continuance of vitality of the typhoid bacillus in running water is the information derived from the fate of the colon bacillus. If this near ally of the typhoid bacillus perishes speedily and in large numbers in a

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given stretch of river there is good reason to suppose that the typhoid bacillus itself will not survive exposure to the same conditions. The rate of mortality among colon bacteria probably indicates more surely than any other factor the death-rate among typhoid bacilli under a similar stress. Wherever we find that an extensive mortality occurs among colon bacteria, in the present state of our knowledge we are justified in assuming that the fatality among typhoid bacteria has been at least equally great.

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