THE LONGEVITY OF *BACILLUS COLI* AND *BACILLUS TYPHOSUS* IN WATER

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It has long been known that in natural water there is a tendency of the intestinal bacteria to die out, but very little is known concerning the conditions which govern their death rate. The available data on the subject deal mostly with streams and reservoirs and are influenced by several variable conditions. Very few data are to be found dealing with pure cultures of bacteria and with known conditions of light, temperature, food supply, dissolved oxygen and the presence or absence of other micro organisms.

Most of the published data on the death rate of bacteria refer to death by drying or by disinfectants. The best work has been by Madsen and Nyman[1], Paul[2], Harriett Chick[3], and Eijkman[4]. Their results show a constant rate of death. When the logarithms of the number of bacteria present are plotted against the time, a fairly straight line results. This is the curve of the monomolecular law and was first noted by Madsen and Nyman and lated used by Harriett Chick, who determined constants for all of her data.

All of our work was done with pure cultures of *B. Coli* and *B. typhosus,* kept constantly fresh and vigorous by daily transfers in one per cent lactose broth. When the culture was to be used, smears were made on agar plates and grown for 12 hours, then suspended in sterile water and suitable portions of the suspension used. This method is better than that of using broth cultures for inoculation as only a minimum amount

1. Madsen and Nyman. Beit. f. Hyg. 57, 388, 1907.
2. Paul. Biochem. Zeit., 29, 202 and 249, 1910.
3. Chick. Jour. of Hygiene, 10, 237, 1910.
4. Eijkman, Verslag. der kon. Akad. Wetenschaffen, V. 21, 1, 510, 1912.

of food material is transferred.

All samples of water used were sterilized before inoculation, the samples kept in hydrogen and nitrogen being in filter flasks, the gas entering through the side tube and escaping through a capillary tube at the top. The other samples were kept in Erlenmeyer flasks.

Table I gives the results of *B. coli* in several waters. The constant k is obtained from the formula:

$$k=\frac{1}{t} \qquad \ln\frac{N}{n}$$

t being time, ln the natural log., N the original number of bacteria and n the final number.

TABLE I.

Death rate of B. coli in various waters.

Hours	Redist. N Free Count	K	Dist. H_2O Count	K	Well Water Count	K	Tap Water U. of I. Count
0	1,104,000		212,000		1,920,000		1,500,000
2	4,000	2.809)	19,000	(1.205)	500,000	.672	2,209,000
12	1,000	.583	16,000	.215	3,000	.528	1,360,000
24	200	.358	200	.290	200	.381	1,280,000
48	5	.256			1	.301	
72			3	.159			
96							2,650,000
6 days							2,400,000
37 days							730,000
Average		.399		.221		.470	

The deep well water was quite high in mineral matter, especially nitrates, but contained only a trace of organic matter.

Table II shows the effect of absence of oxygen on *B. Coli*. The cultures were incubated in atmospheres of nitrogen and hydrogen.

TABLE II.

B. Coli in Air, Nitrogen and Hydrogen

	1						2					
	A		N		H		A		N		H	
Time	Count	K	Count	K	Count	K	Count	K	Count	K	Count	K
0	4,000,000		4,000,000		4,000,000		2,800,000		3,350,000		2,700,000	
1.5	3,100,000	(.169)	2,880,000	.219	2,300,000	.368	1,460,000	.434	2,750,000	(.131)	2,000,000	.200
6	450,000	.364	1,760,000	.137	760,000	.277	57,000	.649	2,250,000	.066	385,000	.324
11	50,000	.398	530,000	.184	28,000	.451	10,000	.469	1,650,000	.059	170,000	.230
24	600	.352	95,000	.155	4,500	.271	3,000	.285	520,000	.077	10,000	.233
36	7	.368	50,000	.121	1,300	.223	1,500	.209	320,000	.065	3,600	.184
48	0	.317	30,000	.102	0	.317			160,000	.063	700	.172
72							960	.111	64,000	.055	400	.108
Aver.		.360		.153		.318		.359		.064		.207

TABLE III.

B. typhosus at different temperatures

	1						2					
	8°		20°		37°		8°		20°		37°	
Time	Count	K	Count	K	Count	K	Count	K	Count	K	Count	K
0	195,00		118,000		110,000		93,000		83,000			
2	10,000	1.485	7,500	1.377	500	2.701			10,000	1.057		
4	2,100	1.035	500	1.365	20	1.806	2,200	.936	800	1.160	sample	
6	1,325	.832	280	1.007	0	1.934	725	.809	1,550	.663		
8	950	.665	230	.779			300	.718	370	.676	contaminated	
10	500	.596	150	.666			265	.586	300	.562		
12			30	.689			130	.548	90	.569		
14	470	.430	30	.591			60	.524	35	.555		
24	60	.336	2	.458			10	.381	0	.471		
Aver.		.768		.866		2.147		.643		.714		

Nitrogen free water was used for the temperature tests. The constants for air are very good but those for nitrogen and hydrogen are variable but always less than for air showing a slower death rate. No reason for this variation was found.

Only a small amount of work was done with *B. typhosus.* The results obtained were very erratic. The technic was the same as was used for *B. Coli.*

It was impossible to obtain good, uniform constants within each set of counts. The averages of different sets were so far apart that no comparison was possible. One possible explanation is that the stock cultures were kept at 20° and the agar plate which was used to furnish the suspension was incubated at 37° for 12 hours. This sudden change may have weakened some cells to such an extent that uniformity was destroyed.

In general, the *B. typhosus* results was similar to those of Whipple and Mayer[1] and Ruediger[2].

The death rate was higher in nitrogen than in air and the rate increased with a rise in temperature.

CONCLUSIONS

Cultures can be kept uniform for several weeks by daily transfers and by keeping them at the same temperature.

In pure, natural water and in redistilled water *B coli* and *B. typhosus* die from starvation in the gradual regular manner observed in other causes of death.

Within the limits of temperature studied, 8° to 37°, the rate of death increased with a rise in temperature.

The death is similar to a chemical reaction and follows the monomolecular law.

The presence of mineral matter has no apparent effect on the organisms.

The presence of oxygen under starvation conditions seems to be harmful to *B. coli* and beneficial to *B. typhosus.*

This work was carried on under the direction of Doctor Otto Rahn of the Bacteriology Department of the University of Illinois and was done in the laboratories of the Illinois State Water Survey.

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1. Whipple and Mayer. Public Health Reports, Vo. 32; Part 2, 76, 1905.
2. Ruediger. Am. J. Pub. Health, 1, 6, 411, 1911.