THE VIABILITY OF *BACT. COLI* AND *BACT. AEROGENES* IN WATER. A METHOD FOR THE RAPID ENUMERATION OF THESE ORGANISMS

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The demonstration by Durham (1900–1), MacConkey (1909), and later by Rogers, Clark and Davis (1914), Rogers, Clark and Evans (1914, 1915), and Clark and Lubs (1915), that the two organisms *Bact. coli* and *Bact. aerogenes* were biologically distinct, differing not only in their biochemical reactions, but also in their natural habitats, has raised many questions of considerable practical importance. It seems quite clear that the presence of *Bact. coli* (low gas ratio, methyl red positive, Voges-Proskauer negative) in water indicates excretal contamination. The significance of *Bact. aerogenes* is, however, less certain. If this organism is a pure saprophyte, living on soil and vegetation, its presence must be of little importance. But if it can be shown that it is commonly present in the mammalian intestine, even though in comparatively small numbers, its occurrence in water immediately assumes significance. If, moreover, it can be shown to survive longer in water than *Bact. coli*, its presence, in the absence of this organism, may indicate an excretal contamination of not very recent date (Gray, 1932).

So far as this country is concerned *Bact. aerogenes* does not appear to be a common inhabitant of unpolluted water. It has, however, been found by a number of workers in human faeces (Cruickshank and Cruickshank, 1931; Gray, 1932; Bardsley, 1934), from which it can frequently be isolated if appropriate selective media are used. Its presence in water must therefore be regarded as at least suggestive of excretal contamination. If its real importance is to be ascertained, it becomes necessary to determine the relative viability of *Bact. coli* and *Bact. aerogenes* in water.

Numerous experiments have been made to determine whether there is any such difference in viability and, if so, what are the factors influencing it.

As early as 1911 Houston reported that in water held in storage there was a change in the ratio of one type to the other. His findings rather suggested that the *aerogenes* type was the less resistant of the two organisms.

These results are not in accord with the findings of later workers. Clemesha (1912) noted that in recently polluted water the ratio of *Bact. coli* to *Bact. aerogenes* was high, but that within a period of 5–15 days there was a reversal of this ratio and the *aerogenes* type became predominant. Rogers (1917) also

reached the same conclusion. In water artificially infected with faeces he observed a gradual change in the ratio of these two types, until at the end of 9 months there were thirty-nine times more *Bact. aerogenes* than *Bact. coli.*

Winslow and Cohen (1918), in a large series of experiments in which tap water was seeded with approximately equal numbers of *Bact. aerogenes* and *Bact. coli* and kept at room temperature in diffuse daylight and in the dark, observed that there was a gradual decrease in the number of viable organisms, and that the *coli* type died off more rapidly than the *aerogenes* type. These authors also recorded a preliminary increase in the number of organisms in two of their experiments, but were of the opinion that it was of no significance and possibly due to the nature of the technique employed rather than to true multiplication. Rector and Daube (1917) a year earlier had also noted this initial increase preceding the gradual decline.

It would thus appear that, under adverse conditions, such as occur in water, there is a gradual dying out of both types, but that *Bact. aerogenes* possesses greater powers of resistance, and is therefore able to survive longer than *Bact. coli.*

In view of the complexity of the factors determining the death of bacteria under natural conditions, and in view of the practical importance of the particular question at issue, it seemed desirable to make further observations on the survival of *Bact. coli* and *Bact. aerogenes* in water, paying particular attention to the effect of temperature.

Exp. I. Using sterilised river water

A 2 litre quantity of Thames river water, collected at Henley in September 1933, was sterilised by autoclaving, and when cool it was inoculated with 0·8 c.c. of a suspension containing both organisms prepared as follows:

Twenty-four hour agar slope cultures of *Bact. coli* and *Bact. aerogenes* were washed off with a small quantity of the sterile river water, and each suspension was standardised by opacity to contain 500 million organisms per c.c. Equal quantities of these two suspensions were then mixed together. Of this mixed suspension 1 c.c. contained approximately 250 millions of each organism and, on the assumption that 50 per cent. of the organisms were viable, 0·8 c.c. was added to the 2 litres of sterile water to give a concentration of approximately 100,000 organisms per c.c. (The subsequent count, however, revealed that only 28·5 per cent. of the organisms were viable.)

After thorough shaking of the suspension a count was made of the viable organisms. The water was then divided into four equal quantities and distributed into sterile flasks, which were closed with sterile cotton-wool plugs, and disposed as follows:

(i) In dark in ice-chest (0° C. to 2° C.).
(ii) In dark in 37° C. incubator.
(iii) In dark cupboard at room temperature (18° C.).

(iv) On bench near window and exposed to diffuse daylight (1 · C.). Viable counts were made on all samples at intervals.

Technique of viable counts

Throughout the work dilutions were made in sterile tap water using 50 drop per c.c. pipettes (external diameter—0·914 mm.) (Donald, 1915, 1916). The roll tube method (see Wilson, 1922) was used, the medium being heart extract agar. The tubes were melted and kept in a 55° C. water bath for about half an hour and transferred to a 45° C. bath 10 min. before using. Three tubes were inoculated from each dilution, the quantity of inoculum being five drops ($\frac{1}{10}$ c.c.). The tubes were incubated for 3 days at 37° C. before counting.

Method of differentiating between types by study of random colonies

To obtain the ratio of the number of *Bact. coli* to *Bact. aerogenes* twenty colonies were picked off into tubes of casein digest broth and incubated for 24 hours at 37° C., and then each was inoculated into Koser's citrate medium and into Eijkman's medium. For inoculating Koser's medium a straight wire was dipped into the broth culture and the minute quantity adhering to the wire seeded into the citrate. For Eijkman's medium a 3 mm. loop was used, one loopful of the broth culture being carried over. The citrate was incubated at 37° C. and the Eijkman at 45° C., this temperature being found preferable to 46° C. Turbidity in citrate with no gas production in Eijkman was taken to indicate *Bact. aerogenes*, and gas production in Eijkman with no growth in citrate to indicate *Bact. coli*. In the few instances where growth occurred in both of the differential media, it was assumed that the colony had been a mixed one and the result was counted as one *coli* and one *aerogenes* type.

(Readings were made at the end of 24 hours with the Eijkman cultures, but 48 hours or longer were required before definite readings of the citrate could be made. The question then arose whether, after such long incubation, *coli* might not be able to grow in citrate. Several experiments were made to determine this, but it was found that even with a comparatively large inoculum there was no danger of *Bact. coli* growing and so affecting the value of the test.)

Results of Exp. I

The results of the viable counts are given in Table I. In all samples, except that kept in the ice-chest, there was a preliminary rise in the number of organisms, this increase being most marked in the sample kept at 37° C., where the phase of active multiplication appears to have continued for over a week. After the first few days, however, and, in the sample kept in the ice-chest from the beginning of the experiment, there was a gradual decline in numbers. The sample at 37° C. became sterile in about 4 weeks. The samples in the ice-chest and in the dark at room temperature showed a gradual but progressive fall. On the other hand, the sample kept in the daylight at room temperature, after behaving for about a fortnight like the sample kept in

the dark, subsequently showed a striking and progressive increase in numbers, till on the 73rd day there were over forty times as many organisms as in the corresponding sample kept in the dark. This observation was very puzzling. The possibility that extraneous saprophytes had been introduced during manipulations was considered, but frequent platings of samples from this flask always gave pure cultures of *Bact. aerogenes* and *Bact. coli*.

After certain other possibilities had been considered, it was realised that the daylight sample had been kept on the bench, where it was liable to be moved from time to time, whereas the cupboard sample had been left undisturbed. Knowing the favourable effect of oxygen on the multiplication and

Table I. *Exp. I. Viable counts on sterile river water inoculated with approximately equal numbers of* Bact. coli *and* Bact. aerogenes. *No. of organisms per c.c.*

Day	Ice-chest	18° C. dark cupboard	18° C. diffuse daylight	37° C.
0	57,000	57,000	57,000	57,000
1	44,000	100,000	130,000	190,000
5	36,000	130,000	97,000	540,000
9	33,000	94,000	88,000	550,000
17	15,000	31,000	83,000	13,000
21	13,000	26,000	120,000	3,200
26	12,000	19,000	120,000	180
30	11,000	12,000	130,000	0
34	13,000	12,000	140,000	0
38	10,000	13,000	150,000	0
45	9,500	9,400	140,000	0
52	7,600	12,000	240,000	0
59	7,400	4,900	340,000	0
66	4,200	6,400	220,000	0
73	3,900	9,700	420,000	0

survival of certain micro-organisms (for references see Wilson, 1930), it was thought that the movement of the daylight flask had led to a degree of aeration of the sample sufficient to bring about a striking increase in the bacterial count.

To test this supposition the contents of the flask kept in the dark at room temperature were divided on the 64th day into two equal quantities, and left under the same conditions, except that one half was shaken vigorously for 10 sec. twice a day, while the other half was left undisturbed. In a count made 2 days later the numbers of viable organisms per c.c. in the non-aerated and aerated samples were 6400 and 18,500 respectively, and after a further 7 days 9700 and 151,000 respectively. The difference in the number of organisms in the two samples was very striking, and suggested that even a slight degree of aeration of water was likely to have a very considerable effect on its bacterial content.

In Table II the results of the differential counts are shown.[1] It will be seen that at the temperature of the ice-chest and at 37° C. there was a rapid

[1] The slight discrepancies between the figures of Tables I and II are due to the fact that in Table II the approximation of the counts to the nearest hundred, thousand or ten thousand was made after the number of *coli* and *aerogenes* had been calculated on the original unapproximated counts.

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Table II. *Exp. I. Results of differential count. Numbers of* Bact. coli *and* Bact. aerogenes, *and ratio of number of* coli *to* aerogenes

	Ice-chest			18° C. dark cupboard			18° C. diffuse daylight			37° C. incubator		
Day	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio
0	27,000	30,000	47 : 53	27,000	30,000	47 : 53	27,000	30,000	47 : 53	27,000	30,000	47 : 53
1	19,000	25,000	43 : 57	42,000	62,000	40 : 60	25,000	110,000	19 : 81	190,000	0	100 : 0
5	21,000	14,000	60 : 40	20,000	110,000	15 : 85	15,000	83,000	15 : 75	540,000	0	100 : 0
9	20,000	13,000	61 : 39	27,000	67,000	29 : 71	8,800	79,000	10 : 90	550,000	0	100 : 0
17	13,000	2,200	86 : 14	11,000	20,000	35 : 65	0	83,000	0 : 100	13,000	0	100 : 0
21	13,000	0	100 : 0	3,900	22,000	15 : 85	6,100	120,000	5 : 95	3,200	0	100 : 0
26	12,000	0	100 : 0	4,700	14,000	25 : 75	6,200	120,000	5 : 95	180	0	100 : 0
30	11,000	0	100 : 0	2,300	9,200	20 : 80	6,700	130,000	5 : 95	0	0	
34	13,000	0	100 : 0	3,600	8,400	30 : 70	13,000	120,000	10 : 90			
38	10,000	0	100 : 0	4,700	8,600	35 : 65	31,000	120,000	21 : 79			
45	9,500	0	100 : 0	470	9,000	5 : 95	7,000	130,000	5 : 95			
52	7,200	380	95 : 5	4,600	7,400	38 : 62	0	240,000	0 : 100			
59	7,400	0	100 : 0	2,500	2,500	50 : 50	17,000	330,000	5 : 95			
66	4,200	0	100 : 0	3,200	3,200	50 : 50	0	220,000	0 : 100			
73	3,900	0	100 : 0	4,900	4,900	50 : 50	0	420,000	0 : 100			

disappearance of *Bact. aerogenes*. In the sample kept at room temperature in the dark, *Bact. aerogenes* predominated during the first 7 weeks or so, after which *coli* and *aerogenes* were present in more or less equal numbers. In the sample kept at room temperature in the daylight *aerogenes* remained predominant throughout. This sample, as has already been explained, was subject to movement, and it would appear as if the resulting aeration had favoured the growth of *aerogenes* more than that of *coli*. This deduction seems to be borne out by a study of the differential count in the aerated and non-aerated portions into which the cupboard sample was divided on the 64th day (Table III). In the non-aerated portion the *coli-aerogenes* ratio remained unaltered, while in the aerated portion *aerogenes* increased to such an extent that *coli* was no longer demonstrable.

Table III. *Exp. I. Results of differential counts made on sample kept at* 18° C. *in dark cupboard after it had been divided into two portions and treated as described in text*

	Non-aerated portion			Aerated portion		
Day	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio	No. *coli*	No. *aerogenes*	*Coli/aerogenes* ratio
66	3200	3200	50 : 50	0	19,000	0 : 100
73	4900	4900	50 : 50	0	150,000	0 : 100

Further evidence in favour of the truth of this explanation is afforded by a study of the results in Exps. II and III (Tables IV and V). In these experiments, in which care was taken to prevent disturbance of the daylight flask, the organisms survived longer at room temperature in the dark than in daylight.

Exp. II. Using raw river water

A second experiment was made, using raw river water naturally contaminated with coliform bacilli.

Two litres of Thames river water at Henley were obtained in October 1933, and the *Bact. coli* and *Bact. aerogenes* content was ascertained in the manner described below. Immediately afterwards the sample was divided into four sterile flasks, and these were kept under the same conditions as in the previous experiment, except that the daylight flask was not disturbed.

Rapid method of estimating Bact. coli *and* Bact aerogenes *content without studying individual colonies*

The common method of enumerating *coli* and *aerogenes* bacilli by plating out from the last dilution of MacConkey broth showing acid and gas, picking off a number of colonies, and testing these in differential media is very unsatisfactory, since, unless the organisms are present in more or less equal numbers, one or the other is apt to be diluted out and the colonies prove to be all of the same type. In Exp. I this fallacy was avoided by plating the

original water directly on to agar. Though this method is satisfactory provided the water contains nothing but coliform bacilli, it is not practicable for raw unsterilised river water, since the differentiation of coliform from non-coliform bacilli is often difficult.

A method was therefore used which has been described by Wilson and his colleagues (1935), to whose report reference should be made for a description of the test and for a general description of the principles underlying the quantitative estimation of coliform bacilli. The test was carried out as follows:

Increasing dilutions (1/10, 1/100, ...) were made in sterile tap water, and from each dilution two tubes of single strength liquid MacConkey medium were seeded, 1 c.c. into each. One tube from each dilution was incubated at 45° C. and the other at 37° C. The production of acid and gas at 45° C. was taken to indicate the presence of *Bact. coli* I, *i.e.* faecal *coli*. From each tube incubated at 37° C. that showed acid and gas formation one tube of Koser's citrate medium was inoculated; if growth occurred, it was assumed that *Bact. aerogenes*, *Bact. cloacae*, or an intermediate type had been present in the corresponding dilution.[1] It was realised that the inoculation of only two MacConkey tubes from each dilution involved a considerable sampling error (Halvorson and Ziegler, 1933), but since our purpose was to compare the general trend of behaviour of *coli-aerogenes* bacilli in water rather than to obtain an exact estimate of their numbers, the method was considered to be sufficiently accurate.

The results given in Table IV show that the organisms survived longest at the temperature of the ice-chest where the predominant type tended to be *Bact. coli*. At room temperature in the dark *Bact. coli* disappeared very early, *aerogenes* alone being present at the end of 5 days. At room temperature in daylight both organisms died out between the 1st and 5th days, while at 37° C. *aerogenes* survived rather longer than *Bact. coli*.

EXP. III. USING RAW RIVER WATER

A third experiment was also made, using raw Thames river water collected at Staines in October 1933, to which equal numbers of *Bact. coli* and *Bact. aerogenes* were added in sufficient concentration to give acid and gas production when 1 c.c. of a 1/10,000 dilution was seeded into liquid MacConkey medium. The ratio of one organism to the other was obtained in the same manner as in Exp. II.

The results are given in Table V. All samples showed a steady decrease in the number of organisms with the early disappearance of *Bact. coli*, except in the sample at ice-chest temperature in which both types were present until the 65th day. The much longer survival of *aerogenes* in the two samples at

[1] In the pages that follow the term *aerogenes* will be used as an abbreviation for the *aerogenes-cloacae*-intermediate group; when the specific organism is meant, the term *Bact. aerogenes* will be used.

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Table IV. *Exp. II. Raw river water—Thames at Henley. Differential estimation of* Bact. coli *and* Bact. aerogenes

Day	Medium and temperature of incubation	Ice-chest				18° C. dark cupboard				18° C. diffuse daylight				37° C. incubator				Type of organism
		Pure	10^{-1}	10^{-2}	10^{-3}	Pure	10^{-1}	10^{-2}	10^{-3}	Pure	10^{-1}	10^{-2}	10^{-3}	Pure	10^{-1}	10^{-2}	10^{-3}	
0	Liq. MacConkey at 37° C.	AG	AG	AG	–	AG	AG	AG	–	AG	AG	AG	–	AG	AG	AG	–	Coliform
	,, ,, 45° C.	AG	AG	AG	–	AG	AG	AG	–	AG	AG	AG	–	AG	AG	AG	–	faecal *coli*
	Koser's citrate medium	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	*aerogenes*
1	Liq. MacConkey at 37° C.	AG	AG	AG	–	AG	AG	–	–	AG	AG	–	–	AG	AG	–	–	Coliform
	,, ,, 45° C.	AG	AG	AG	–	AG	AG	–	–	AG	AG	–	–	AG	AG	–	–	faecal *coli*
	Koser's citrate medium	+	+	–	–	+	+	–	–	+	+	–	–	+	+	–	–	*aerogenes*
5	Liq. MacConkey at 37° C.	AG	AG	–	–	AG	AG	–	–	–	(10 c.c.)		–	AG	(10 c.c.)		–	Coliform
	,, ,, 45° C.	AG	AG	–	–	–	–	–	–	–	(10 c.c.)		–	–	(10 c.c.)		–	faecal *coli*
	Koser's citrate medium	–	–	–	–	+	–	–	–	–	–	–	–	+	(10 c.c.)			*aerogenes*
9	Liq. MacConkey at 37° C.	AG	AG	–	–	AG	(10 c.c.)		–	–	–	–	–	–	(10 c.c.)		–	Coliform
	,, ,, 45° C.	AG	AG	–	–	–	–	–	–	–	–	–	–	–	–	–	–	faecal *coli*
	Koser's citrate medium	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	*aerogenes*
14	Liq. MacConkey at 37° C.	AG	–	–	–	–	(10 c.c.)		–	.	.	.	.	–	–	–	–	Coliform
	,, ,, 45° C.	AG	–	–	–	–	–	–	–	.	.	.	.	–	–	–	–	faecal *coli*
	Koser's citrate medium	–	–	–	–	–	–	–	–	.	.	.	.	–	–	–	–	*aerogenes*
18	Liq. MacConkey at 37° C.	AG	(10 c.c.)		–	–	–	–	–	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	(10 c.c.)		–	–	–	–	–	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	(10 c.c.)		–	–	–	–	–	.	.	.	.	.	.	.	.	*aerogenes*
22	Liq. MacConkey at 37° C.	AG	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	*aerogenes*
29	Liq. MacConkey at 37° C.	AG	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	(10 c.c.)		–	.	.	.	.	.	.	.	.	.	.	.	.	*aerogenes*

Table V. *Exp. III. Raw river water—Thames at Staines, to which approximately equal numbers of* Bact. coli *and* Bact. aerogenes *were added. Differential estimation of the two organisms*

Day	Medium and temperature of incubation	Ice-chest					18° C. dark cupboard					18° C. diffuse daylight					37° C. incubator					Type of organism
		Pure	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Pure	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Pure	10^{-1}	10^{-2}	10^{-3}	10^{-4}	Pure	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
0	Liq. MacConkey at 37° C.	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	Coliform
	,, ,, 45° C.	AG	AG	AG	AG	–	AG	AG	AG	AG	–	AG	AG	AG	AG	–	AG	AG	AG	AG	–	faecal *coli*
	Koser's citrate medium	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	*aerogenes*
1	Liq. MacConkey at 37° C.	AG	AG	AG	AG	–	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	Coliform
	,, ,, 45° C.	AG	AG	AG	AG	–	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	faecal *coli*
	Koser's citrate medium	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	*aerogenes*
5	Liq. MacConkey at 37° C.	AG	AG	AG	AG	AG	AG	AG	AG	AG	–	AG	AG	AG	AG	–	AG	AG	–	–	–	Coliform
	,, ,, 45° C.	AG	AG	AG	AG	AG	–	–	–	–	–	AG	AG	–	–	–	AG	AG	–	–	–	faecal *coli*
	Koser's citrate medium	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	+	–	–	–	*aerogenes*
9	Liq. MacConkey at 37° C.	AG	AG	AG	AG	–	AG	AG	AG	AG	–	AG	AG	AG	AG	AG	AG	AG	–	–	–	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	faecal *coli*
	Koser's citrate medium	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+	+	+	–	–	–	*aerogenes*
16	Liq. MacConkey at 37° C.	AG	AG	AG	–	–	AG	AG	AG	–	–	AG	AG	–	–	–	AG	–	–	–	–	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	faecal *coli*
	Koser's citrate medium	+	+	+	–	–	+	+	+	–	–	+	+	–	–	–	+	.	.	.	.	*aerogenes*
24	Liq. MacConkey at 37° C.	AG	AG	AG	–	–	AG	–	–	–	–	AG	AG	–	–	–	–	–	–	–	–	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	faecal *coli*
	Koser's citrate medium	+	+	+	–	–	+	–	–	–	–	+	+	–	–	–	–	–	–	–	–	*aerogenes*
30	Liq. MacConkey at 37° C.	AG	AG	AG	AG	–	AG	(10 c.c.)		–	–	AG	–	–	–	–	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	+	+	+	–	+	–	–	–	–	+	–	–	–	–	.	.	.	.	.	*aerogenes*
37	Liq. MacConkey at 37° C.	AG	AG	AG	–	–	AG	(10 c.c.)		–	–	AG	(10 c.c.)		–	–	.	.	.	.	.	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	+	+	–	–	+	–	–	–	–	+	–	–	–	–	.	.	.	.	.	*aerogenes*
44	Liq. MacConkey at 37° C.	AG	AG	–	–	–	AG	(10 c.c.)		–	–	AG	(10 c.c.)		–	–	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	AG	–	–	–	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	+	–	–	–	+	–	–	–	–	+	–	–	–	–	.	.	.	.	.	*aerogenes*
51	Liq. MacConkey at 37° C.	AG	–	–	–	–	AG	(10 c.c.)		–	–	–	(10 c.c.)		–	–	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–	.	.	.	.	.	*aerogenes*
58	Liq. MacConkey at 37° C.	AG	(10 c.c.)		–	–	AG	(10 c.c.)		–	–	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	AG	(10 c.c.)		–	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	+	–	–	–	–	+	–	–	–	–	.	.	.	.	.	.	.	.	.	.	*aerogenes*
65	Liq. MacConkey at 37° C.	AG	(10 c.c.)		–	–	AG	(10 c.c.)		–	–	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	–	–	–	–	–	+	–	–	–	–	.	.	.	.	.	.	.	.	.	.	*aerogenes*
72	Liq. MacConkey at 37° C.	–	(10 c.c.)		–	–	AG	(10 c.c.)		–	–	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	–	–	–	–	–	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	–	–	–	–	–	+	–	–	–	–	.	.	.	.	.	.	.	.	.	.	*aerogenes*
79	Liq. MacConkey at 37° C.	.	.	.	.	.	–	(10 c.c.)		–	–	.	.	.	.	.	.	.	.	.	.	Coliform
	,, ,, 45° C.	.	.	.	.	.	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	faecal *coli*
	Koser's citrate medium	.	.	.	.	.	–	–	–	–	–	.	.	.	.	.	.	.	.	.	.	*aerogenes*

room temperature was very striking, but it must be noted that approximately ten times as many *aerogenes* as *coli* were present at the commencement of the experiment.

Discussion

Exp. I, made with sterile water to which known numbers of *Bact. coli* and *Bact. aerogenes* were added, confirms on the whole the findings of previous workers. The preliminary increase in the viable count in three of the samples indicates that, for the first 24 hours at least, the multiplication rate exceeded the death rate. After the first few days there was a gradual decline in the viable counts, the decrease being most rapid at 37° C.

So far as the viability of the two types is concerned, reference to Table II indicates the importance of the temperature factor. At the temperature of the ice-chest and at 37° C. conditions were more favourable for survival of *Bact. coli* than *Bact. aerogenes*. At room temperature there was no such marked difference, but the results strongly suggested that the conditions for survival were more favourable to *Bact. aerogenes* than to *Bact. coli*.

The second and third experiments were performed, using raw unsterilised river water in order more closely to simulate natural conditions. In Exp. II the initial numbers of coliform organisms were rather small, and it is not surprising that three of the samples became sterile within a fortnight. The striking difference in the differential counts noted in Exp. I was not observed. *Bact. coli* died out at about the same rate as *aerogenes* at 0° C. in the dark and at 18° C. in daylight, while at 18° C. in the dark and at 37° C. *aerogenes* survived rather longer than *coli*.

In Exp. III the initial numbers of coliform organisms were higher than in Exp. II, but still somewhat below those in Exp. I. The rate of death, however, was more rapid than in Exp. I, all of the samples being sterile, so far as coliform bacilli were concerned, within 80 days. This confirms the findings of previous workers in showing that these organisms survive longer in sterile than in non-sterile water. The differential counts showed a much longer survival of *aerogenes* than of *Bact. coli* in both of the room temperature samples, and a slightly longer survival in the sample kept at 37° C. At 0° C. the two organisms survived for about the same length of time. In judging these results it must, however, be remembered that *aerogenes* was present originally in numbers about ten times as high as those of *coli*.

Taking all these experiments into consideration, it seems clear that on the whole *aerogenes* is more resistant than *Bact. coli*, particularly at ordinary atmospheric temperatures. At 0° C. *coli* is perhaps slightly more resistant than *aerogenes*, while at 37° C., at any rate in raw water, the reverse probably holds true.

SUMMARY AND CONCLUSIONS

1. The survival of coliform organisms was studied in river water, either raw or sterilised, kept at different temperatures.

2. For determining the coliform count and the differential *coli-aerogenes* count in sterilised river water, direct plating of the water on agar, with subsequent study of a number of colonies picked at random, was used. For raw river water the rapid method described by Wilson and his colleagues (1935) was used, which obviates the necessity of plating and of colonial examination.

3. When *Bact. coli* and *Bact. aerogenes* were held in stored river water, which was protected from agitation, they underwent a gradual decrease in numbers and finally disappeared. At 37° C. they died out rapidly, but survived for a much longer time at temperatures in the neighbourhood of 0° C. They were able to survive longer in sterile water than in raw water.

4. Observations, however, made on water kept at room temperature and subjected to gentle aeration showed that not only did the organisms not die out, but that they actually multiplied, so that their numbers were considerably higher at the end of two months than at the beginning of the experiment.

5. In raw river water coliform bacilli survived longer at room temperature when kept in the dark than in daylight.

6. On the whole, *aerogenes*[1] proved more resistant than *Bact. coli* to the environmental conditions provided. This was particularly noticeable in samples kept at room temperature (18° C.). In samples of raw water kept at 37° C. *aerogenes* proved slightly more resistant than *coli*, while at 0–2° C. the reverse was true.

7. The general conclusion seems to be that, except at very low temperatures, *aerogenes* is likely to survive longer in raw river water than *Bact. coli*.

8. This conclusion is clearly of importance in the interpretation of the *coli-aerogenes* results in water analysis.

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[1] See footnote on p. 443.

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